BIOLOGICAL LIMITATIONS TO THE PRODUCTION OF PROCESSED BROCCOLI IN TASMANIA

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SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF TASMANIA (MAY 2009)

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This project has been facilitated by Horticultural Australia (Project VG06051) in partnership with Simplot Australia and the University of Tasmania's Post Graduate Research Scholarship scheme. It has been funded by voluntary contributions from industry. The Australian Government provides matched funding for all HAL's research and development activities.

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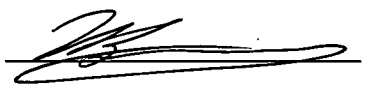

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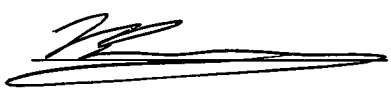

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ABSTRACT

An increased pressure from international competitors on the domestic production of processed broccoli has meant that this sector needs to identify ways in which it can reduce the cost of raw produce supplied to local processors. This is best achieved through improvements in net yield and efficiencies that reduce labour costs. This thesis addresses three biological impediments to achieving improvements in these areas: Uneven harvest maturity resulting in numerous harvests; processing inefficiencies introduced by inappropriate head shape; and reduced quality from hollow stems.

Investigations undertaken during the course of this work have found that the variation in the meristem diameter during inflorescence initiation explains 50% of the variation in maturity encountered at harvest. This finding highlights the importance of introducing and maintaining seedling uniformity during the transplant and establishment phases of production to minimise variation in maturity at harvest, thus reducing harvest costs. It also provides a basis for future research to focus on techniques to achieve this outcome.

The study has also demonstrated the influence of head shape on processing efficiency and net yield. The more compact shape of 'Shamrock' resulted in a superior (low) ratio of the less valuable stem tissue to the more valuable floral tissue when compared to the elongated form associated with 'Marathon'. The architecture of the 'Shamrock' inflorescence also provided higher total floret yield, and more opportunity to manipulate the levels of stem material to suit seasonal requirements. The attributes associated with 'Marathon' resulted in greater processing efficiency, with the comparatively open branch structure of this variety producing more segments within factory specification, and a smaller proportion that required re-dicing. These findings establish the significant impact of head shape on net yield and processing efficiency, providing an additional tool to improve these through varietal selection.

Since the early 1930's the development of hollow stems in broccoli and cauliflower has been variously attributed to boron deficiency or to factors associated with plant growth rates. The data presented in this study provides evidence that the

development of hollow stem in broccoli is related to growth rate when manipulated by planting density. Evidence is also provided to show that mechanical tissue stresses generated in the stem during inflorescence development can lead to the development of stem fractures. Tissue extensibility tests revealed higher levels of radial, tangential and longitudinal extensibility in the outer pith and vascular / cortex tissues, while the central pith tissues were less extensible in all dimensions. Circumferential tension in the transverse plane was also found to be stored in the vascular cortex region. It is proposed that the differential mechanical properties and capacities for growth across the stem tissues may cause sufficient internal strain to cause the fracture of the less extensible and possibly weaker tissue of the central pith.

The findings of this thesis have established a fundamental understanding of the physiological mechanism underlying the development of hollow stem, the importance of the transplant and establishment phases of production on head uniformity, and the significant influence of head shape on net yield and processing efficiency. Application of this knowledge by the Tasmanian broccoli processing industry will assist in improvements in net yield and provide opportunities for efficiencies that reduce labour costs.

ACKNOWLEDGMENTS

"I want creation to penetrate you with so much admiration that wherever you go, the least plant may bring you the clear remembrance of the Creator. ...One blade of grass or one speck of dust is enough to occupy your entire mind in beholding the art with which it has been made. "

— St. Basil the Great (329-379)
Bishop of Caesarea, Cappadocia

Our knowledge of the world we live in may have surpassed that of what was known in the 4th century AD, but the knowledge gained has only increased our understanding of its sheer complexity, the inherent brilliance of its design, and should leave us in greater awe than that with which St. Basil the Great viewed it, regardless of our philosophical positions. While it is unpopular for a scientist to espouse a belief in God, I do so, with a conviction that has only firmed as I have encountered the astounding brilliance contained within the life of a plant. And so it is with deep gratitude that I thank the Creator, for the opportunity to explore His creation.

I am deeply appreciative of the continued generosity, encouragement, mentoring, guidance and wise advice provided to me by my supervisors Dr Alistair Gracie and Dr Phil Brown during the course of my candidature. Throughout this collaboration I have been stimulated and inspired intellectually, challenged to write a logical story with academic rigour, and have been saved on more than one occasion from the jaws of statistical calamity. Writing a thesis is time consuming, even for supervisors, and I sincerely appreciate the time you have made available to me, and the significant personal commitment you have both made to my personal development and the completion of this degree. Thank you.

To my gorgeous, loving and faithful wife Julia, and my infinitely lovable children Nicholas, Ashleigh and Olivia, thank you for your loving support and the freedom to pursue my dreams at unibersity (sic), particularly in light of the sacrifices you have made along the way on my behalf. The good news is that Dad will no longer

be locked in his room working on his broccoli. The bad news is you will still have to eat it (it's good for you!).

Thanks Mum for the love and support along the way, and for the genes. They were foundational to the completion of this degree. Dad would be proud!

Thanks also to my friends who likely haven't understood my obsession with broccoli (I suspect some may think an asylum would have been a more appropriate institution to house me), but have still followed my progress over the last few years, and provided support and encouragement along the way.

Thanks also to:

Dr Frank Hay and Dr Sarah Pethybridge for your generosity in allowing me access to equipment and supplies in the lab, and other assistance provided along the way.

Julie Overall and Jayne Mollema for the provision of excellent, enthusiastic and egalitarian administrative assistance and support.

David Stirling, Petra Novak, Chris Russell and Kim Davis from Simplot Australia for your assistance in providing direction and feedback for this project and assistance in the conduct of the experimental work undertaken in the field and factory.

Dr Richard Rawnsley and Keith Pembleton (F.B.S.) for the statistical advice and discussions.

Vaughan Beer, Nat Donaldson, Mat Costello, Scott Carlson, Craig Morris, Lyndon Head, Leon Hingston, Craig Palmer, Gordon Tuck, Phil Gardam, Craig Priest, Colin Harvey, Shaun Suitor, Stephen Ridge, Lyndon Butler and Vaughan Trebellico for your assistance in maintaining experimental plots, data collation and lab work.

I am also indebted to the organisations that have funded this project and my scholarship, Simplot Australia, Horticultural Australia Ltd, and the University of Tasmania's Post Graduate Research Scholarship scheme.

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CHAPTER 1

INTRODUCTION AND THESIS SCOPE

IMPORTANCE OF BROCCOLI AS A VEGETABLE CROP

Until early last century broccoli (*Brassica oleracea* L. var. *italica* Plenck) was a relatively unknown crop on a global scale, only grown extensively in Italy and to a lesser degree in Britain (Gray, 1982). Since then, production and consumption of broccoli has spread to most parts of the world, with the popularity of both broccoli and cauliflower increasing to the extent that 19 million metric tonnes was grown across 87 countries in 2006 (Food and Agriculture Organisation of the United Nations, 2008). Broccoli is produced in Australia for both the fresh and processing markets, with most fresh produce sold in domestic markets due to its limited post-harvest life, while processed product is destined for both domestic and export markets. In part, the increased popularity of broccoli and cauliflower in Australia and internationally is based on the *Brassica* vegetables improved reputation as a health food. Plants in this genus produce glucosinolates, flavonoides, polyphenols, vitamins, fibre and pigments that are active in preventing the development of various types of cancer (Park and Pezzuto, 2002).

As Australian consumers are becoming more aware of broccoli's health benefits, consumption is increasing, with each Australian currently eating an estimated 2.4 kg each year (Ausveg, 2006). Consequently the production of broccoli harvested nationally rose from 26,155 t in 1992 to a plateau of 55,000 t during 2003-2005 (Figure 1). The area planted out to broccoli also increased from 4,582 ha to 7,621 ha during this period (ABS, 1998-1999, ABS, 2000-2008). In 2005-06 domestic production of broccoli was valued at $ 90.4 M (ABS, 2007). During the peak production period of 2002-2005 Tasmania produced 14.4 – 18.7% of Australia's broccoli (ABS, 1998-1999, ABS, 2000-2008). While other regions of Australia grow for the fresh market, the majority of broccoli grown in Tasmania is for processing.

In 2004-05, a total of 2,700 t was produced in Tasmania for the domestic fresh market while 7,400 t of product was grown for the processing sector (Anon., 2005).

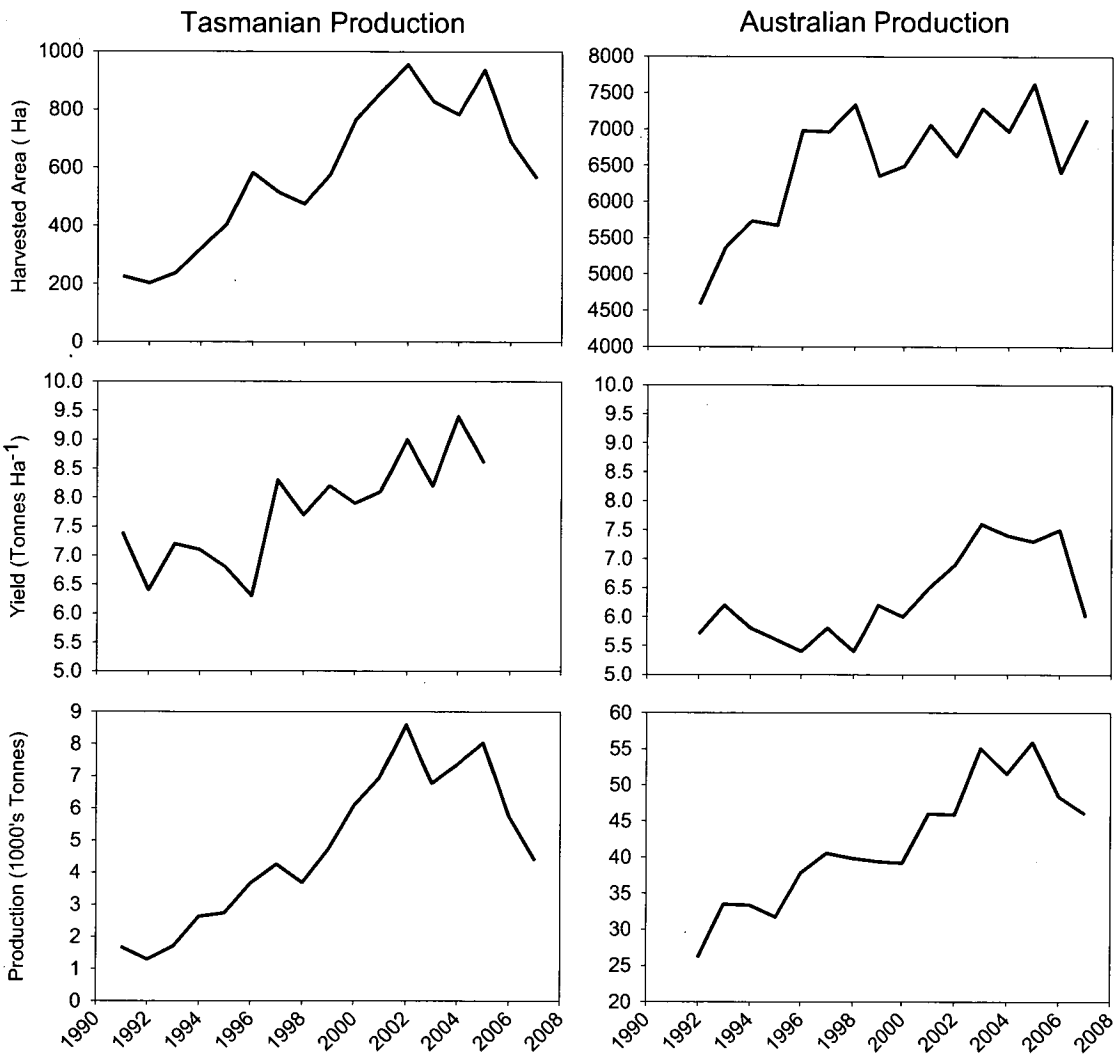


Figure 1. *Production statistics for both fresh market and processed broccoli grown in Australia and Tasmania from 1991 to 2007, (ABS, 1998-1999, ABS, 2000-2008).*

While the production of broccoli in Australia increased during the decade leading up to 2005, the volume of frozen processed vegetable product imported into Australia also increased. While New Zealand remains a major exporter of frozen produce into Australia, China's influence has been steadily increasing, with the value of Chinese product increasing by 424% between 2002 and 2006 to a total value in 2006 of $10 M (James, 2006). Not only is China able to provide mixed vegetable products direct to supermarket shelves, they also have the capacity to supply diced raw produce at competitive prices for packaging by local processors.

The continued rise in imports of frozen mixed vegetables and minor vegetables has had a marked impact on domestic broccoli production (Apted et al., 2006), and from 2005 to 2007 Australian production dropped 10 000 t (ABS, 1998-1999, ABS, 2000-2008).

The desire to locate processing facilities close to both a local regional market and raw produce led to the regionalisation of the processed vegetable industry, with processing facilities concentrated in key production areas (Apted et al. 2006). These key production areas such as the north of Tasmania are now heavily exposed to international competition. Of the five major vegetable processing companies that dominate global production: Unilever, Simplot, McCain Foods, ConAgra and DelMonte (Apted et al., 2006), two of these, Simplot Australia and McCain Foods, operate vegetable processing facilities on the North West Coast of Tasmania. The availability of cheaper raw produce from other countries has led to price reductions as these multi-national processors attempt to rationalise their production costs to reflect world markets. The resulting decrease in the gross value of product (Figure 2), also driven by the recent increase in domestic production (Apted et al., 2006), has meant that farmers are reluctant to accept contracts. Consequently the production of broccoli in Tasmania has been almost

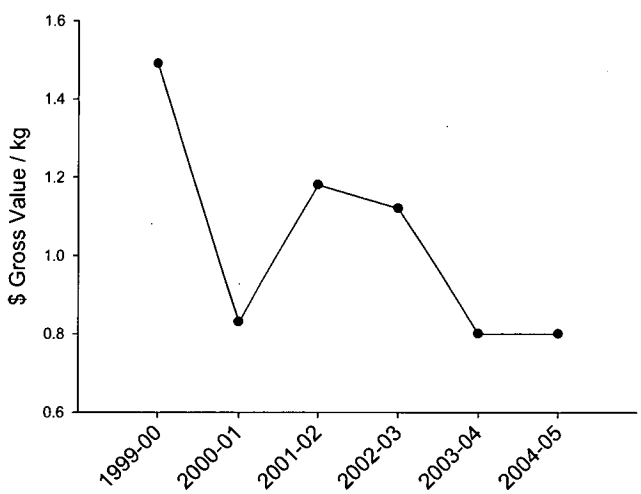


Figure 2. *Gross value of processed and fresh market broccoli 1999-2000 to 2004-05 (Anon., 2005; citing ABS, cat.# 7503.0 & 7121.0).*

halved from 8035 t in 2005 to 4380 t in 2007 (ABS, 2000-2008) this representing 36% of the national decline in broccoli output over the same period. Although official figures are not detailed, the shortfall in the availability of raw broccoli is presumably being supplemented by vegetable imports into Tasmania from China, totalling $4 M in 2007 (Department of Foreign Affairs and Trade, 2008).

The trend of increasing international pressure on domestic production is likely to continue unless pivotal underlying issues are addressed (Anon., 2005). A factor of key importance is the cost of raw vegetable produce. While the capital required to establish processing facilities is similar worldwide, their location is primarily determined by their proximity to the production area, proximity to the domestic market, and competitive pricing for raw produce (Apted et al., 2006). These first two requirements are satisfied in Australia by the location of processing facilities on the North West Coast of Tasmania where, until recently, the cost of local produce has been internationally competitive. However, the disparity in labour costs between Australia and other developing nations has become increasingly important as countries like China continue to build their export capacity, and are able to export frozen, processed broccoli at locally competitive prices. There is a massive gap between Australian labour costs ($33 $hour^{-1}$; including on costs) and that of China ($1.27 $hour^{-1}$) (Anon., 2005), and it is this gap that is in part compensating for the logistical costs associated with exports. Not only is this product cheap, it has been diced to the appropriate size by hand, is of high quality, and requires minimal processing before being included in frozen food products (David Stirling, pers. com.) While the short term consequence of comparatively high priced local raw produce is a reduction in local contract prices and production, the long term consequence of failing to reduce the unit cost of production is likely to be the relocation of local facilities to other regions. It is therefore imperative to the survival of the industry in Tasmania that the unit cost of raw produce is addressed.

The likely avenues for achieving a reduction in the cost of raw produce, apart from direct action on labour costs, are through efficiency gains attained by increasing the scale of production units, and increases in yield (Anon., 2005, Apted et al., 2006). Net yield can be improved through changes that maximise the quality of

the raw produce, whilst gross yield can be enhanced by improved agronomic practices and varietal changes. Consequently the manner in which processed broccoli is produced has been heavily scrutinised by industry.

PROJECT SCOPE

The processed broccoli industry in Tasmania is facing a major challenge from low cost overseas producers. Increased output and efficiency in production, along with reduced production costs, are required for the industry to ensure its continued survival. A task-force has been established in Tasmania to examine strategies such as direct seeding, new varieties and mechanical harvesting in an attempt to reduce labour costs and improve net yield. This project complements the task-force effort by investigating three biological barriers to improving the labour costs, yield and quality of processed broccoli.

UNDERSTANDING UNIFORMITY IN HARVEST MATURITY

Tasmania has relatively high labour costs in comparison to countries such as China that compete within the global processed broccoli market (Anon., 2005). Any areas of production and processing that involve use of labour can therefore make a significant contribution to production costs of the final processed product. Broccoli grown for both the fresh and processed markets is cut by hand in the field, loaded into plastic crates and trucked to the factory. This practice involves a number of tractors, trucks and a significant labour component. These costs, and those associated with organisation of the harvest, make a significant contribution to the high cost of the raw produce in Tasmania.

The harvested component of a broccoli plant is the immature inflorescence, and as an actively growing and developing structure of the plant, it is only at a growth stage suitable for harvesting for a short period of time. This window is referred to as harvest maturity. While individual plants are only harvestable during a limited window of opportunity, broccoli crops typically exhibit uneven harvest maturity,

meaning that different plants are ready to harvest at different dates. This uneven spread in maturity necessitates 3-5 cutting events spread over a week or more, which in turn exacerbates the costs of the harvest operation. This variability in harvest maturity could be attributed to differences in the physiological stage of individual plant development, and to differences inferred by the field staff using what are currently subjective assessment techniques.

Improvements in harvest uniformity will reduce harvesting costs and improve processing efficiency by improving on the reliability of supply of raw produce to the processing factory, preventing unnecessary downtime created by fluctuations in supply. Crop uniformity is also a prerequisite for once over harvesting which is required for the development of mechanical harvesting, a development that would significantly reduce labour requirements and therefore cost of the raw material for processing.

Variability in harvest maturity within a crop may be introduced at the time of floral evocation, through plants initiating inflorescences at different times, or by different rates of inflorescence development after initiation. Developing an understanding of the underlying factors that influence the timing of inflorescence initiation and the rate of its development may provide the key to providing more even harvest maturity, and the development of objective assessment techniques. Factors such as germination and establishment rates if direct seeded, seedling evenness if transplanted, crop husbandry, genetic and environmental factors are all likely contributors to the timing of harvest maturity.

Despite several agronomic studies examining effects of various treatments on harvest uniformity in broccoli crops (Chung, 1982, Marr, 1985, Salter et al., 1984), little is known about the plant developmental events that contribute most to plant to plant variability or the points in the crop lifecycle where the variability is introduced. Given the large number of factors (genetic, agronomic and environmental) that combine to produce variation in harvest maturity, the development of a management strategy to reduce variability is not possible without identification of the developmental events or stages that contribute most to crop variability. This project aims to address this deficiency by identifying the

stage, or stages, of transplant crop development during which the variation is introduced. Identifying the key stages of crop development that contribute to variation in harvest maturity of transplanted crops will allow future research to focus on these at strategic points in the crop life cycle.

UNDERSTANDING HEAD SHAPE

While reduction of labour costs during production through increased uniformity of harvest maturity may lead to reduced raw material costs, the quality of the raw material will also impact on the competitiveness of the industry by affecting wastage rates and processing efficiency in the factory. One of the quality attributes of the raw material is the shape of the harvested inflorescence (commonly referred to as the head). Broccoli used in the processing market must be transformed from an entire head into stem and higher order branch segments suitable for inclusion into frozen vegetable mixes and other pre-packaged products. The process must efficiently and reliably produce diced segments of consistent size and high quality. Genetic and environmental variation in head shape and the architecture of internal branching influence the efficiency of the processing environment. Yet strategies to manage head shape to improve processing efficiency have not been developed as very little research defining the ideal head shape and examining factors influencing shape has been undertaken.

The harvested inflorescence is a heavily branched structure with branches terminating in multiple flowers. The most valuable parts of the inflorescence are the segments of higher order branches with floral parts attached (referred to as florets), with factory specifications for florets generally restricting marketable product to specific size ranges. The remaining stem tissue on the harvested inflorescence (referred to as core) may be utilized as a lower value component by dicing for inclusion in frozen vegetable mixes, or may be treated as waste material from the processing operation. The ratio of core to floret segments, typically ranging from 0.13 – 0.73, is used as a key measure of processing efficiency, with a high ratio being undesirable. The architecture of the head may influence this ratio,

leading to the possibility of processing productivity gains if head architecture can be manipulated to consistently deliver higher quality raw material.

In addition to improved productivity from the raw material through management of head shape, gains in processing efficiency may also be possible. The processing operation involves removal of the high value florets from the core, and aims at maximising the yield of florets fitting factory specifications for size and appearance. The internal branch architecture will influence the size distribution of the segments produced during processing. Florets that do not meet factory specification, being over or under size, must be recut to achieve size specifications or be rejected. This secondary dicing adds further costs to the processing chain and tends to produce blocky, less attractive floret segments, while cutting of undersized sections increases wastage rate.

Consequently, variability in external shape and internal branch architecture that leads to undesirable head structure can increase labour requirements during processing and contribute to additional losses through wastage. While the primordial arrangement of branches is produced in a highly organised fundamental spiral, it is likely that other aspects such as internodal distance and the number of branches produced will be influenced by genetics and environmental factors. Increased understanding of head shape and how architectural parameters affect processing efficiency, particularly the core:floret ratio and the size distribution of processed florets, will enable efficiency gains and improvements in net yield.

UNDERSTANDING HOLLOW STEM

Hollow stem, a physiological disorder of broccoli, is a second factor affecting quality of the harvested raw material. Hollow stem is a disorder of both broccoli and cauliflower and is characterised by the formation of longitudinal cavities that traverse significant portions of the stem. In Tasmania, up to 80% of a crop may be affected. These cavities often extend through the region of the stem cut during harvest, leaving the cut product prone to disease or the ingress of soil, neither of which are attractive to consumers. Additionally tissue bordering the cavities may

also become necrotic prior to harvest. This is a particularly important factor for fresh market broccoli as it significantly decreases visual appeal of the product to consumers, resulting in affected product being of low quality or unmarketable. The presence of hollow stem in processed broccoli is also detrimental as the core of hollow stem affected material cannot be used for dicing to include in frozen product mixes, resulting in downgrading of the material from low value product to waste.

Hollow stem was first described in the early 1930's (Dearborn and Raleigh, 1935, Chupp and Horsfall, 1933). Since these early trials, scientific and popular publications have attributed hollow stem in cauliflower and broccoli to both boron deficiency and other factors such as high growth rate and nitrogen application. The weight of evidence provided by these studies would seem to favour growth rate or related factors as the driving force behind this disorder yet boron is commonly recommended by agronomists in popular literature as a remedy for hollow stem in both broccoli and cauliflower.

Identifying the underlying mechanism of the hollow stem disorder in broccoli has a two-fold benefit. Firstly, understanding the underlying physiological mechanism would provide a real opportunity to develop new treatments of greater efficacy. Treatments reducing the incidence and severity of hollow stem would provide substantial savings through increased net yield and the reduction of processing waste associated with hollow stem. Secondly, if a mechanism other than boron deficiency is identified as the driving physiological mechanism, material and logistical costs associated with the application of boron could be reduced, thus again contributing to a reduction in the cost of raw produce.

PROJECT OBJECTIVES

In summary, to complement industry efforts to reduce the cost of raw produce through efficiency gains and improvements in net yield, this project has three main objectives. Firstly, this project aims to identify the time of at which most harvest variation is being introduced to transplanted broccoli crops. Discovering the key

crop stages at which this variation is introduced will provide a strategic focus for future research aimed at achieving a once over harvest in broccoli. Secondly, this project aims to determine the influence of external head morphology and internal branch architecture on the key measures of processing outcomes (Core: floret ratio and appropriate segment sizing). Identifying these influences will provide further tools with which processors can assess the current impact of head shape on processing efficiency and provide an additional framework for the evaluation of new varieties. Thirdly, this project aims to identify the physiological mechanism responsible for the occurrence of hollow stem in broccoli. As hollow stem disorder also has a direct influence on quality and net yield, a mechanistic understanding of its development will improve the chances of developing effective measures to address this issue, thus reducing the financial impact of this disorder on the cost of raw produce.

CHAPTER 2

BROCCOLI INFLORESCENCE ARCHITECTURE

INTRODUCTION

The objective of broccoli processing is to separate the higher order branches (floral portion) of the inflorescence from the stem. Ideally, a high proportion of the segments derived from this process will fall within specific size limits. The external morphology and internal branching structure, or architecture, of the broccoli inflorescence is an important attribute that has a marked influence on this process. These architectural attributes are determined by the ontogenetic pattern of branch development and floral initiation. Both the orientation and the inter-nodal distances at which higher order branches appear are important as they determine the size of the floral bearing segments (metamers) derived during processing.

The efficiency of broccoli processing can be measured using two key parameters; the ratio of stem material to branch segments bearing floral tissue (core:floret ratio) and the proportion of segments that meet target size specifications. The core:floret ratio determines net yield, and a low ratio is generally desired as the floret component is the most saleable component of the inflorescence. A high percentage of floral segments in the specified size range is also desirable as processed floral segments that are oversized require further downstream processing, and acutely undersized sections are wasted, increasing labour costs and reducing efficiency.

Whereas the machinery used to segment broccoli for the processed market has been designed to achieve maximum throughput while efficiently separating the stem from the floret metamers, it is a relatively inflexible system. In particular, blade trajectories are generally fixed, resulting in the same cutting angles and

distances being used to process populations of broccoli heads that inevitably vary in size and architecture. In this context, variability in head shape and the internal branching structure have a significant impact on the core to floret ratio and the production of uniformly sized segments.

To illustrate this, in the locally used AEM Decora® (AEM Machinery Ltd., NE Lincolnshire, England; Figure 1), heads are first inverted and placed in a plastic dish that forms part of the conveyance system.

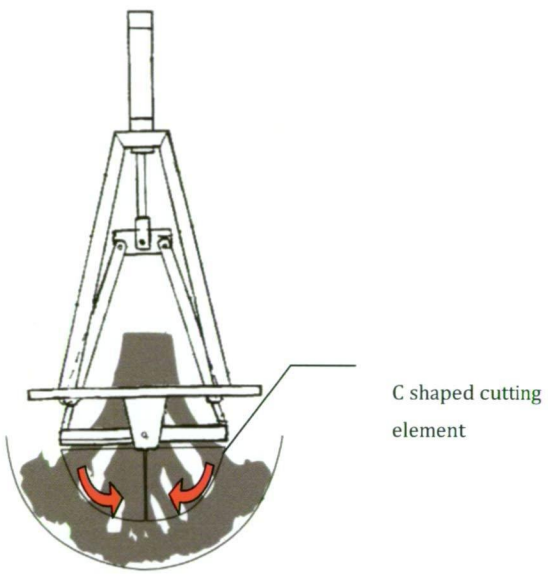


Figure 1. *Illustration of the cutting assembly used in the AEM Decora machinery for segmentation of broccoli and cauliflower inflorescences. Diagram modified from (Ellis, 2000).*

The heads are held in place by spikes projecting upwards from the bottom of the dish while being moved under the cutting apparatus. While resting in the dish, two C shaped cutter elements then descend over the core and close together, inscribing a parabolic trajectory that separates the stem from the floret segments. The stem, now held by the cup formed by the two C shaped cutter elements, is then ejected from the machine while the floret segments flow out underneath on a conveyor belt. The stability of the head in the dish is affected appreciably by the curvature of the inflorescence surface. Heads with a higher degree of curvature (Figure 2B & C) will have an increased tendency to rock out of alignment if not held sufficiently by the spikes, whilst those with lower degree of curvature will tend to bridge across

the bottom of the bowl (Figure 2A). In both of these instances, the trajectory of the blade through the desired portion of the head will be altered. The ideal head would have a curvature matching that of its receptacle, producing maximum stability and ensuring that all of the marketable floret material is removed from the stem.

Assuming that the heads are of equal external dimensions and a curvature suiting the receptacle, the blade will consistently pass through the same region of the head (Figure 2). While this will ensure efficient separation of florets from stems, variation in the internal branch structure of the heads will still influence segmentation. In broccoli, the branch order though which the blade passes is the primary determinant of the size of the metamer produced. The lower the branch order, the larger the segment. Thus changes in the inter-nodal distances for branches of the different orders will influence which order the cutting blade passes through.

If the changes in branching pattern such as increased elongation mean the blade passes though higher order / position branches, a greater portion of smaller segments will be produced. Conversely, in a more compact branching structure, the blades would more likely travel though lower order / position branches, producing proportionally more large segments (Figure 2D). Thus, the external shape and internal branching architecture have a significant impact on final output and quality.

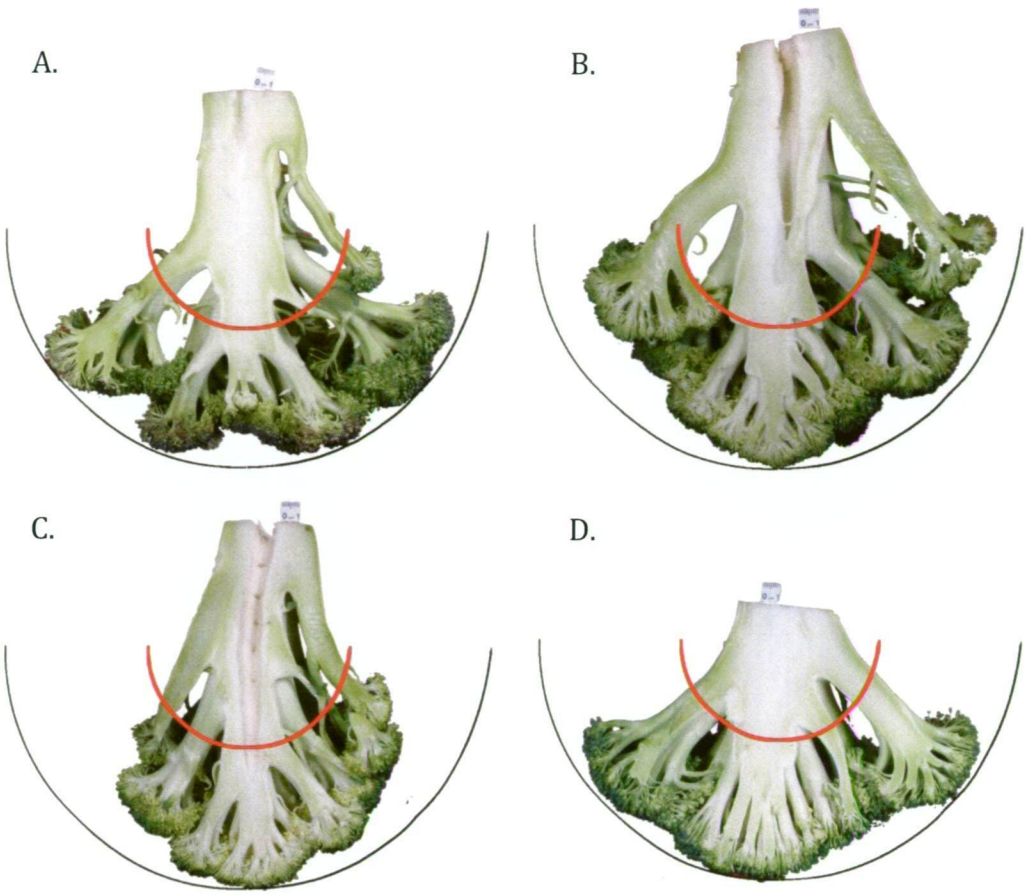


Figure 2. *Simulated influence of head architecture on segmentation using a blade of constant trajectory. Each head has been halved radially through the lowest branch. Representative blade trajectory in red. Receptacle in black. Elements in the drawing are to scale.* ***(A)*** *'Marathon' head shape with open branch structure and curvature flatter that the receptacle.* ***(B)*** *'Marathon' head shape with curvature greater than the receptacle, high skirt depth, long stem and a dense branch structure.* ***(C)*** *'Marathon' head shape with a long stem, a high degree of curvature and narrow diameter. Both B and C would be susceptible to rocking and produce a large amount of core.* ***(D)*** *The more compact 'Shamrock' head shape with an uneven surface but still well fitted to the receptacle. This head has a lower inter-nodal distance between II order branches, a more compact branch structure, and is likely to produce a large terminal segment.*

MORPHOGENESIS AND HEAD SHAPE

The architecture of an inflorescence is determined by the pattern of branching in conjunction with the timing and location of floral structures (Benlloch et al., 2007). The branching pattern is determined by which meristems give rise to shoots before higher order meristems take on a floral identity. Hence the fundamental shape of the broccoli inflorescence is largely determined by its morphogenesis and

the genetic control of this process. An understanding of inflorescence morphogenesis, including knowledge of the aspects that are tightly controlled by genetics and those that are open to environmental influence, provides the foundation for establishing which aspects of head architecture can be manipulated during crop production and those that can only be managed through variety selection.

The components of the broccoli and cauliflower inflorescence are initially produced as primordia on the flanks of the apical inflorescence meristem in a generative spiral. As further primordia are produced, these II order primordia become themselves inflorescence meristems and produce their own primordia in a generative spiral on their own flanks. This progression continues up to the 10^{th} order in Cauliflower (Smyth, 1995) leading to the development of a curd mass composed of up to 1×10^7 inflorescence meristems (Kieffer et al., 1996). In contrast, this process is halted much earlier in broccoli, with the meristems taking on floral identity after a relatively short period of curd thickening (Kieffer et al., 1998). This self-repetition leads to the well recognised fractal nature of these brassica crops, where each smaller sub-unit mirrors the structure of its parent. This self-repetition can even be seen in the surface of the head, which is itself a large hemi-sphere made up of smaller hemi-spheres. In broccoli however, the process of self-repetition is limited, ceasing once floral organogenesis commences. The morphogenesis of the broccoli inflorescence from the vegetative stage through to buttoning is described in detail by Kieffer et al. (1998) and Tan et al. (1998).

The creation of branch and floral primordia in a generative spiral leads to the development of a Fibonacci phyllotactic system. The underlying fundamentals that lead to this phenomena are rudimentary to an understanding of the architecture of inflorescence branching. Additionally, the parameters used to measure a Fibonacci phyllotaxis are in effect some of the parameters that determine the shape of the inflorescence.

In a compressed spiral of leaves or other organs, each leaf is generally in contact with two other leaves lower down in the spiral, and these referred to as *contacts*. As the eye follows these two contacts, numerous apparent spirals, rotating both

right and left, and at a steeper pitch than the genetic spiral, can be seen. These apparent spirals are referred to as *contact parastichies* (Mitchison, 1977, Turing, 1992). Contact parastichies dissect their common primordia at 90° (Callos and Medford, 1994).

The Fibonacci sequence is a numerical sequence elucidated by Leonardo Fibonacci in 1202 while investigating population growth in rabbits. This sequence is defined by

$$Fn+1= Fn + Fn-1$$

producing the series 0,1,3,5,8,13,21,35,56,91 ..... where the next term in the sequence is defined by the sum of the previous two terms. Interestingly, the number of right (m) and left (n) parastichies in a spiral phyllotaxis correspond to terms within the Fibonacci sequence (Callos and Medford, 1994). As an example, broccoli and cauliflower inflorescences have been described as having a 5+8 phyllotactic arrangement, having 5 right and 8 left handed parastichies (Kieffer et al., 1998, Sadik, 1962).

In the analysis of plant architecture, the phyllotactic arrangement of a species can be defined by three constraints (Callos and Medford, 1994).

1. *Order of organ formation*
   The generative order of primordial formation can be derived by measuring primordial diameters. The largest organs are generally the oldest, although this does not hold true for all species. The order of formation can also be determined using vascular development, or in the absence of this, displacement of the youngest primordia from the shoot apical meristem (Callos and Medford, 1994).

2. *Divergence angle (DA)*
   The divergence angle is the smallest angle formed between two successive primordia in the genetic spiral and the centre of the SAM, determined by the apical centre of symmetry. In the case of a leaf

primordium, the centre of developing vascular tissue is considered to be the centre of symmetry. A number of estimates should be used to calculate the DA due to variability within the spiral. This variability is exacerbated in low order Fibonacci systems (Callos and Medford, 1994). In a phyllotaxis with parastichies following the Fibonacci sequence, the divergence angle (DA) between successive primordia approximates the golden angle, *phi*, 137.5°. As the number of parastichies increase, the departure of the DA from *phi* decreases (Mitchison, 1977).

3. *Plastochron Ratio (PR)*

   The plastochron ratio is a measure of the rate of meristem expansion as given by the ratio between radii of successive organ initials, the radii being measured from the centre of the apical meristem. The radius of the oldest primordium is the numerator. As the diameter of the apical meristem increases, the pitch of the genetic spiral decreases (Mitchison, 1977).

Richards (1951) found that the PR of successive Fibonacci systems increased at a stable rate, leading him to define the phyllotaxis index (PI).

$$(PI) = 0.38 - 2.39 \log \log(PR)$$

Each set of parastichies in the Fibonacci sequence has a characteristic PI, allowing the phyllotactic pattern to be defined using the divergence angle and plastochron ratio. As plant organ arrangements are known to move through different Fibonacci phyllotaxis as growth and development occurs, the PI provides a tool for tracking these changes (Callos and Medford, 1994).

As morphogenesis proceeds the inflorescence passes first through the 1:2 then 2:3 and 3:5 parastichy systems before stabilising at a 5:8 Fibonacci phyllotaxis until harvest (Kieffer et al., 1998). This change in parastichy number and hence the phyllotaxis index (PI) indicates that plastochron ratio (rate of meristem

expansion) is declining as the meristem increases in diameter (Callos and Medford, 1994). As the diameter of the apical meristem increases, the pitch of the genetic spiral decreases (Mitchison, 1977), and thus inter-nodal distances of the II order branches should decrease with progress towards the top of the inflorescence.

The key element contributing to the shape of the broccoli and cauliflower inflorescence is the tightly controlled placement of the branch primordia in a generative spiral. As for most spiral phyllotaxis, Kieffer et. al. (1998) reported the divergence angle for primordia of orders I-IV approximated the Golden Angle (φ), 137.508. This precise rotation of each new primordia provides the basis for the self repetition seen in both broccoli and cauliflower.

Of next importance is the iteration interval (ITI), the number of primordia formed before primordia of the next order arise on their flanks. The iteration interval determines the number of Order +1 branches held by each limb of the inflorescence. As meristematic activity occurs for a longer period of time in older primordia, they therefore produce more branch primordia before the branch primordial themselves become meristematic in nature. Kieffer *et al.* (1998) observed that the ITI for the production of III order branches was 11-12 II order primordia in broccoli but greater for cauliflower (15-16) and romanesco (20-21). This iteration interval appears to be relatively constant in cauliflower however changed between orders in broccoli when measured on a 'mature' inflorescence. The instability in the ITI is possibly attributed to the onset of floral meristem identity. At this juncture, all meristems except for the I order axis cease inflorescence meristem production. The I order meristem continue to produce II order branches (position ca. 30 onwards) which then themselves assume a floral identity, initiating floret primordia (Kieffer et al., 1996).

The relative rate of primordia production (RRP) between orders will alter the number of branches of the next order carried by an inflorescence stem. If primordia of the higher orders form at a slower rate, an inflorescence of lower complexity and different form will develop. If these primordia form at a higher rate, a more complex and compact head shape will develop. Kieffer et al. (1998)

found the RRP production of III order branches to be stable at 1:1 for the first 30 I order positions.

The timing and the location at which the inflorescence meristems assume floral identity also contributes to head shape. The older (lower positioned) primordia, or those last to switch identity will not only bear more branches (Kieffer et. al. 1998) but will have greater diameters (Callos and Medford, 1994). They may also have undergone more cell division along their axis, allowing for greater branch length once cell expansion occurs. It is immediately evident that in broccoli, for the lowest positioned II order branches to bear their florets within the floret dome, greater inter-nodal distances (branch lengths) are required, and that inter-nodal elongation must vary according to order and position. The continued II order meristem production for positions 30 onwards whilst the other meristems are undergoing floral transition most likely allows for the filling of the 'crown' of the inflorescence.

Thus the placement of primordia in a spiral phyllotaxis, the lag between the development of the next highest order primordia (ITI), the relative rate of primordia production between orders, the temporal and spatial dynamics with which the primordia switch identity, and finally the differential elongation of the branches by position and order, all combine to determine the architectural form of the inflorescence. The precise patterning, timing and regularity involved would seem to indicate that it is a highly orchestrated organogenesis controlled by plant genes (Hardwick, 1984).

GENETIC ORCHESTRATION OF THE BROCCOLI INFLORESCENCE

The basic shape of the broccoli and cauliflower inflorescences is established by events that are controlled by plant genetics, and in at least one study, head shape descriptors have been shown to differ significantly between genotypes (Tan et al., 1999a). The homeotic genes involved in the development of plant inflorescences are best understood, albeit not completely, in *Arabidopsis thaliana* and *Antirrhinum majus*. Of the homeotic genes identified for *Arabidopsis*, a *Brassicaceae* relative,

homologues have been found in cauliflower and broccoli for APETALA 1 (AP1) , APETALA 2 (AP2), APETALA 3 (AP3), CAULIFLOWER (CAL) , FRUITFUL (FUL), LEAFY (LFY), UNUSUAL FLORAL ORGANS (UFO) and TERMINAL FLOWER 1 (TFL1) (Kempin et al., 1995, Carr and Irish, 1997, Duclos and Bjorkman, 2008). The discovery that the mutant gene *cal-1* in association with the *ap1* mutant produced an abnormal cauliflower type inflorescence in *Arabidopsis* (Bowman et al., 1993) led to a series of studies that tried to elucidate the role of these genes in determining the different phenologies observed within *Brassica oleracea*.

The molecular and genetic models so far proposed have not provided an adequate explanation for the observed difference in broccoli and cauliflower phenotypes from that of *Arabidopsis* (Labate et al., 2006, Duclos and Bjorkman, 2008). Floral homeotic gene expression continues during inflorescence development in broccoli, even during the floral arrest stage, and no homeotic mutations appear in the subsequent floral structures. While the *cal-1* and *ap1* mutants do produce a cauliflower type inflorescence with abnormal flowers in *Arabidopsis*, cauliflower and broccoli themselves produce normal floral structures (Kempin et al., 1995). Additionally, the alleles for BoCAL and BoAP1 in cauliflower and broccoli appear to explain only a small proportion of the observed differences in phenotype (Labate et al., 2006), and BoCAL which is nearly fixed, is also found in *B. oleracea* var. acephala, (kale) and the wild type *B. oleracea* var. *oleracea* (wild cabbage), other members of the group with again quite distinct phenotypes (Purugganan et al., 2000). A more recent study has measured the expression of BoAP1-a, BoAP1-c, BoCAL, BoFUL-a, BoFUL-b, BoFUL-c, BoFUL-d, BoLFY, AP2, UFO, BoTFL1 and the cauliflower curd-specific genes CCE1 and BoREM1 in heads at different stages of development (Duclos and Bjorkman, 2008). The pattern of gene expression observed was different to that of *Arabidopsis* and was inadequate to explain the cauliflower and broccoli phenotypes. The authors of this study concluded an explanatory model must include other genes.

An understanding of the genetic control of the broccoli inflorescence development would provide an invaluable breeding tool to alter its architectural attributes to suit the processing environment. Our current understanding of the genetic orchestration of inflorescence development in headed brassica's suggests that the

genetic mechanism is more complex than originally thought (Purugganan et al., 2000), and that the tools to achieve genetic manipulation are still futuristic.

ENVIRONMENTAL INFLUENCES ON HEAD SHAPE

While there is a strong genetic influence over inflorescence development, plants often have a phenotypic response to changing environmental conditions. The phenotypic response in broccoli is unlikely to be limited to the vegetative phase, and both primordial inflorescence development and relative growth rates of the inflorescence tissues are likely to respond to environmental stimuli.

All plants use both changes in total solar energy and in the ratio between red and far red light (R:FR) to detect the proximity and actual shading of nearby plants (Ross et al., 2005). In plants that employ a shade avoidance response, a reduction in the R:FR typically includes increased internodal extension, early flowering and an increase in apical dominance, which in turn suppresses axillary branch development (Smith, 1997). Leaf angle is also known to change in response to low light levels, leaves being borne at higher angles under this condition (Whitelam and Johnson, 1982). Changes in any of these parameters could conceivably have some impact on the structural development of the broccoli inflorescence. Assuming the effect of low light level on the floral infrastructure is similar to that of other plant parts, low light levels could increase the internodal distances and vertical orientation of branches within the inflorescence leading to the development of taller head shapes. As the production of multiple meristems in the shoot apex of broccoli is likely to involve reduced apical dominance, an increase in apical dominance in the broccoli inflorescence brought about by low R:FR might also result in a less complex structure with limited branching.

Light quality and quantity, and also the availability of moisture and nutrients is influenced by plant competition. Increasing planting density leads to greater competition between plants for these resources, and changes in crop canopy structure in response to this might elicit changes in the structure of the inflorescence. While increased plant density does reduce the size of the

inflorescence (Damato, 2000, Wurr et al., 1992), its reported effect on shape is varied. Some studies have observed that flatter heads are produced at densities below 200, 000 plants ha^{-1} (Salter et al., 1984) while in other studies significant changes in head shape have not been observed (Chung, 1982).

Agronomic studies have indicated that extreme temperature can play a role influencing the shape of the broccoli inflorescence and that this effect is mediated by the stage of development. While a temperature of -3 to -5 °C during inflorescence initiation resulted in death of the shoot apex, at buttoning (*ca.* 10mm diameter), subzero temperatures of -5 °C altered bud colour, evenness and clustering in the cultivars 'Marathon' and 'Fiesta'. Head shape however, was only affected in 'Fiesta', this variety producing flatter heads at this temperature (Tan et al., 1999b). The external morphology of the inflorescence in broccoli also becomes distorted at higher growing season mean (GSM) temperatures while minimum GSM appears to have limited influence. The occurrence of concavity or an undulating surface is exhibited at maximum GSM temperatures less than 18.4-21 °C and greater than 22-26.8°C. The threshold at which this response occurs is dependent on genotype (Dufault, 1996).

Although there is an apparent interaction between variety and temperature (Tan et al., 1999a, Dufault, 1996), all researchers seem to agree that genotype appears to have more sway over head shape than environmental variables.

MEASUREMENT OF HEAD SHAPE

Head shape is often assessed in variety selection trials (Chowings, 1974) or in experiments concerned with the manipulation of factors that may alter the quality of the harvested head (Chung, 1982, Tan et al., 1999a). It can also be used in the selection of produce for export (Tan, 1999). While legislation can be a source of well defined descriptors for produce quality, there are no such definitions of head shape for broccoli in Australian law, although some brief descriptors have been published by the Victorian Department of Natural Resources and Environment (O'Donnell et al., 1998). The form of the inflorescence is commonly measured on a

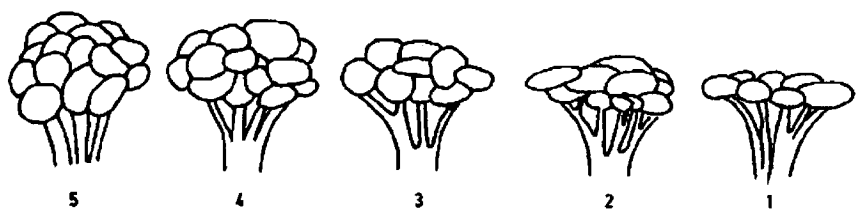
subjective scale using a graphic key or verbal description that includes parameters such as diameter, curvature, branching angle and clustering / lumpiness (Darling et al., 2000, Chung, 1982, Tan et al., 1999b, Dufault, 1996). Darling et. al. (2000) used a 5 point head curvature scale to assess the susceptibility of heads to bacterial soft rot, establishing that heads with lower curvature were more susceptible to infection. With the exception of diameter which is easily measured in discreet units, the keys are commonly 5 point scales based on that of Chowings (1974). The key used by Chung (1982) in assessing broccoli cultivars at different densities for once over harvest is included below (Figure 3) and is representative of other scales in the literature.

(a) *Spear size* – The spear diameter and the butt diameter are measured in mm.
(b) *Bud size* – The largest bud of each spear is measured in mm.
(c) *Evennness of bud size*

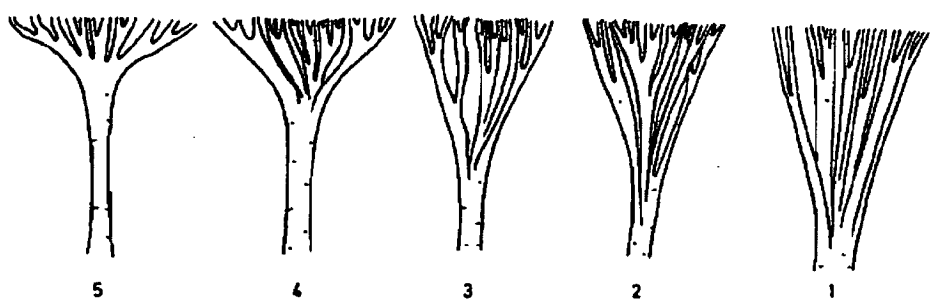
Description	*Rating*
All buds the same size	5
75% of buds the same size	4
50% of buds the same size	3
25% of buds the same size	2
less than 25% of buds the same size	1

(d) *Cluster separation of the head*

Description	*Rating*
No obvious bud cluster	5
Bud clusters only on small part of head	4
Bud clusters only on perimeter of head	3
Bud clusters on 75% of head surface	2
Bud clusters over all surface of head	1



(e) *Head shape* – Diagrammatic representation of terminal spear showing deep heads (5) and shallow heads (1).



(f) *Branching* – Diagrammatic representation of branching within terminal spears showing wide angle branching (5), narrow angle branching (1).

Figure 3. *Broccoli head quality assessment key used by Chung (1982).*

CONCLUSION

Primordial development of the inflorescence, such as placement of the primordia in a spiral phyllotaxis, appears to have a significant genetic component that is unlikely to be altered by environmental influences. Yet other processes such as the iteration interval, the relative rate of primordia production, and the timing of floral identity could be influenced by fluctuations in light, temperature or other environmental variables during primordial development. For instance, low light levels could alter these parameters to bring about changes in the plastochron ratio and larger internodal distances, or different rates of elongation between branch orders, resulting in longer or flatter heads as the plants tries to place its flower buds in a better position to receive sunlight. While the fundamental shape of the head appears to be set during primordial development, the shape of the head might also be influenced by external factors during tissue expansion and secondary growth of the inflorescence branches. Again variability in environmental conditions such as light, temperature and soil moisture could interrupt or alter inflorescence development during this phase.

If environmental factors do alter head shape within a particular genotype, then both site differences and agronomic practices are likely to play a role in altering key processing requirements such a segment size distribution and possibly the core:floret ratio. Given the strong genetic role in inflorescence development, cultivar selection is also likely to play a significant, if not the strongest role, in altering these outcomes.

CHAPTER 3

THE INFLUENCE OF BROCCOLI HEAD SHAPE ON PROCESSING EFFICIENCY

INTRODUCTION

In addition to colour, size and general appearance, the external shape and internal architecture of the inflorescence are of specific importance to processed broccoli. During processing, broccoli heads are cut into stem (referred to as core) and higher order branch segments. Both components are included in frozen vegetable products. The higher order segments, which bear the floral parts, are referred to as florets and are the most valuable part of the inflorescence. The core, while able to be used in mixed vegetable products, is viewed as less attractive and required in lower quantities, making this a less valuable component. The inherent structure of the broccoli inflorescence means that the quantity of core harvested often exceeds factory requirements with the excess material becoming waste product. Consequently, the ratio of core to floret segments (by fresh weight) is a key measure of net yield and processing efficiency, with a low ratio being most desirable. External shape and branching architecture are likely to influence this ratio, but it is unknown to what extent these characteristics can be manipulated during the growing of the crop.

Both the floret and core components must fit within the factory size specifications. While the core material lends itself to this process, producing floret segments of uniform size is much more challenging. This is because the machinery used in the segmenting process provides a limited set of blade trajectories and, at present in commercial operations, is unable to adjust to individual head dimensions or internal branching structure. Typically only one blade trajectory is used to process an entire crop. Segment size is therefore principally determined by branch architecture and external curvature which influences the stability of the head when being cut. The inherent variability in both external shape and internal

branching of the harvested produce results in an uneven distribution of floret segment size. Oversized segments require further downstream dicing, creating less attractive blocky segments, while also increasing labour costs and decreasing processing efficiency.

While variation in the architecture of internal branching and external shape is likely to influence processing outcomes, very little research has been published in this area. The fundamental architecture of the inflorescence is determined by genotype (Kieffer et al., 1998, Benlloch et al., 2007), however it is unknown to what extent environmental and agronomic practices influence this expression in broccoli. The objective of this study was to determine if site factors influence inflorescence architecture and examine the influence of the broccoli inflorescence architecture on processing efficiency.

METHODOLOGY

CROP SURVEY

A survey of 9 commercial broccoli crops was used to examine the intra- and inter-crop variation in plant inflorescence architecture. All the crops used in this survey were grown for the processing market and consisted of the cultivar Marathon. The crops were established using transplants at the 4-5 leaf stage on farms located in the main production regions in Tasmania. Crops were planted at commercial densities of ca. 33 000 plants ha^{-1} in two rows per bed (1.64m tractor wheel centres), from the 18th January to 7th February 2006. Twenty randomly selected plants were sampled during the second commercial harvest of each crop from an 8 bed wide section that had been marked out as representative of the paddock prior to crop establishment. Crops were sampled at the second harvest cut (the second sequential harvest event) to ensure that an even representation of maturity between sites was used. Each head was cut on the underside of the lowest branch insertion of the inflorescence, weighed and then photographed in plan view using a Power Shot G5 digital camera (Canon Inc.) mounted on a stand to maintain a consistent height and viewing angle. The head was then dissected longitudinally,

cutting through the 1st position II order branch, and a side elevation image was taken of each half. Scale markers were placed in each plane of measurement for calibration of image analysis measurements. Image analysis was used to assess head diameter (mm), head height (mm), skirt height (mm), stem projection (mm; the distance the stem projected past the skirt), eccentricity (e) and the branching angle of the II Order position 1 branch ($\acute{\alpha}$) (Figure 1).

DECORA EFFICIENCY

To examine the influence of inflorescence architecture on processing efficiency heads from two cultivars, Marathon and Shamrock, with distinct differences in morphology were processed using factory equipment. Thirty newly harvested intact heads of each cultivar from the same production site were randomly sampled from Simplot Australia's refrigerated factory bins on 14th April 2007. Heads were selected for harvest based on a diameter greater than 100mm and less than 200mm, floral beads sizes of less than 3mm, and compactness. Stems were trimmed to the underside of the lowest branch insertion of the inflorescence. Digital images were taken from plan and side elevation views of the intact head. Scale markers were again placed in the planes of measurement used during image analysis. Image analysis was used to assess head diameter (mm), head height (mm), skirt height (mm), stem projection (mm) and eccentricity (e). Heads were then individually segmented using an AEM Decora® floretting machine, set at a 70mm depth and using 122mm dial knives. Stem and floret segments from each plant were retrieved, placed in a plastic bag and again refrigerated before individual segments were assessed.

The physical measurements taken of each segment included stem fresh weight (g), stem length (mm), total number of floret segments, orthogonal floret diameter (mm) and floret length (mm). For each stem, branch residue was recorded by starting at the II order branch position 1 and rotating in the direction of the generative spiral, recording clean cuts and remnant branches by order and position. The core:floret ratio was calculated as the proportion of the stem fresh weight to total floret fresh weight of the entire inflorescence.

Processing efficiency was characterised by rating floret length and orthogonal diameter dimensions against factory specifications. Florets with at least one dimension exceeding the maximum length or diameter were classified as oversize whilst those that exceeded the minimum specification were regarded as undersized. Florets meeting the specification for both dimensions were recorded

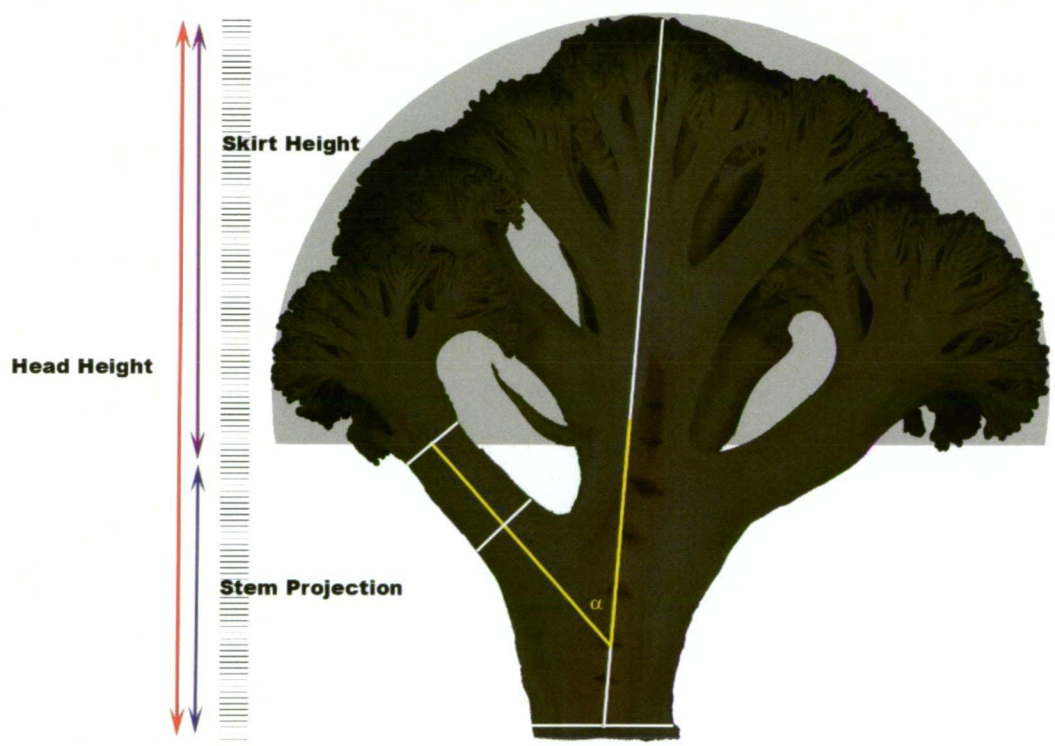


Figure 1. *The descriptors used to express head shape were head height (red), skirt height (purple), stem projection (blue) and angle of the lowest inflorescence branch (yellow). Curvature was estimated as the eccentricity of the ellipse (grey) that best described the curvature needed by a receptacle to fit the head.*

as on target. Broccoli heads that inadvertently tipped over during segmentation by the Decora floretting machine were not included in the analyses.

IMAGE ANALYSIS

Digital images were prepared for analysis in Adobe Photoshop CS (Adobe Systems Inc.) and analysed using the Fovea Pro 4.0 plug-in (Reindeer Graphics Inc). Traits measured included head diameter (mm), head height (mm), skirt height (mm), stem projection (mm), eccentricity (curvature) and branch angle of the I order position 1 inflorescence branch. All dimensions computed were calibrated using scale markers.

The head diameter (d) was calculated from the 2-dimensional surface area of the head

$$d = 2(A/\pi)^{0.5}$$

where A = area and π, the ratio of a circles circumference to diameter. Head and skirt heights were measured from one half of the longitudinally dissected head, using the y dimension of a bounded rectangle extending from the dorsal surface of the head to the excision point or lowest part of the skirt respectively.

To calculate eccentricity, an ellipse that best estimated the arc of a receptacle needed to fit the curvature of the head was fitted around the side elevation images of the inflorescence perimeter. The moment angle (orientation) and maximum and minimum axis lengths were measured from this ellipse using Fovea Pro. For a horizontal ellipse, eccentricity (e) was calculated as:

$$e = \frac{\sqrt{a^2} - b^2}{a}$$

and for a vertical ellipse as:

$$e = \frac{\sqrt{b^2} - a^2}{a}$$

where a = maximum axis / 2 and b = minimum axis / 2.

STATISTICAL ANALYSIS

Statistical analysis was conducted using SPSS version 16.0. Sums of squares was used to partition variation within and between sites using one-way ANOVA for each of the different head shape descriptors across the nine sites. Where data from the Decora efficiency trial met the assumptions of normality and equality of variance a univariate generalised linear model was used to establish varietal differences between architectural and processing parameters. Where these assumptions were not met, a non parametric Kruskal Wallace or Mann-Whitney U test was used. Linear regression models and associated statistics were computed using the SPSS linear regression option.

RESULTS

VARIABILITY IN HEAD SHAPE ACROSS SITES

The median values and variation for each of the head shape descriptors used were calculated for each of the nine crops surveyed (Figure 2). Median head diameters ranged from 115 mm (Crop 5) to 184 mm (Crop 8) and with the exception of Crop 5, the majority of heads for all crops were all within the 100 – 200 mm factory specification for harvest size. Median head heights ranged from 106 mm (Crop 5) to 188 mm (Crop 4) with a significant proportion of heads being longer than the factory specifications (140-150mm). Skirt height medians ranged from 73 mm (Crop 5) to 115 mm (Crop 8). The median head curvature, measured as eccentricity (e) was lowest at 0.37 (Crop 7) and highest at 0.57 (Crop 9) with the

distribution at most sites skewed toward rounder heads. Almost all heads had a higher degree of curvature (*e*) than the receptacle (0.75) of the Decora floretting machine. Median stem projection in some sites contributed as little as 31 mm (Crop 5) to total head height while in other crops as much as a 78 mm (Crop 4) contribution was made. The median angle of the lowest inflorescence branch ranged from 34° (Crop 4) to 49° (Crop 1).

Partitioning of the variation using sum of squares demonstrated greater variation within than between crops for all of the architectural descriptors (Table 1). Only 7% and 17% of plant-to-plant variation in branch angle and eccentricity could be explained by site, respectively, whereas site differences accounted from 32 to 38% of the variation for head dry weight, head diameter, head height and skirt height.

Table 1. *Percentage variation in head shape characteristics which could be attributed to within and between sites using sums of squares.*

Shape Descriptor	Variation Between Sites (%)	Variation Within Sites (%)
Head Diameter	32	68
Head Dry Weight	38	62
Head Height	34	66
Skirt Height	36	64
Stem Projection	27	73
Eccentricity	17	83
Branch Angle	7	93

Head dry weight, width, and diameter were all strongly correlated. Skirt width and stem projection, which together make up total head height, were respectively strongly and moderately correlated with head dry weight, width and diameter. Branch angle was negatively correlated with stem projection and head height, although these relationships were respectively moderate and weak (Table 2). Eccentricity was weakly correlated with stem projection but was not significantly correlated with any other head shape descriptor.

Table 2. *Correlation matrix of 'Marathon' head shape descriptors (n = 174).*

	Head D^{wt}	Head Diameter (mm)	Head Height (mm)	Skirt Height (mm)	Eccentricity	Stem Projection (mm)
Head Diameter (mm)	0.896**					
Head Height (mm)	0.812***	0.831***				
Skirt Height (mm)	0.839***	0.827***				
Eccentricity	0.095	0.122	0.149	-0.011		
Stem Projection (mm)	0.520***	0.560***		0.368***	0.245***	
Branch Angle (ά)	-0.027	0.047	-0.335***	0.015	-0.113	-0.545***

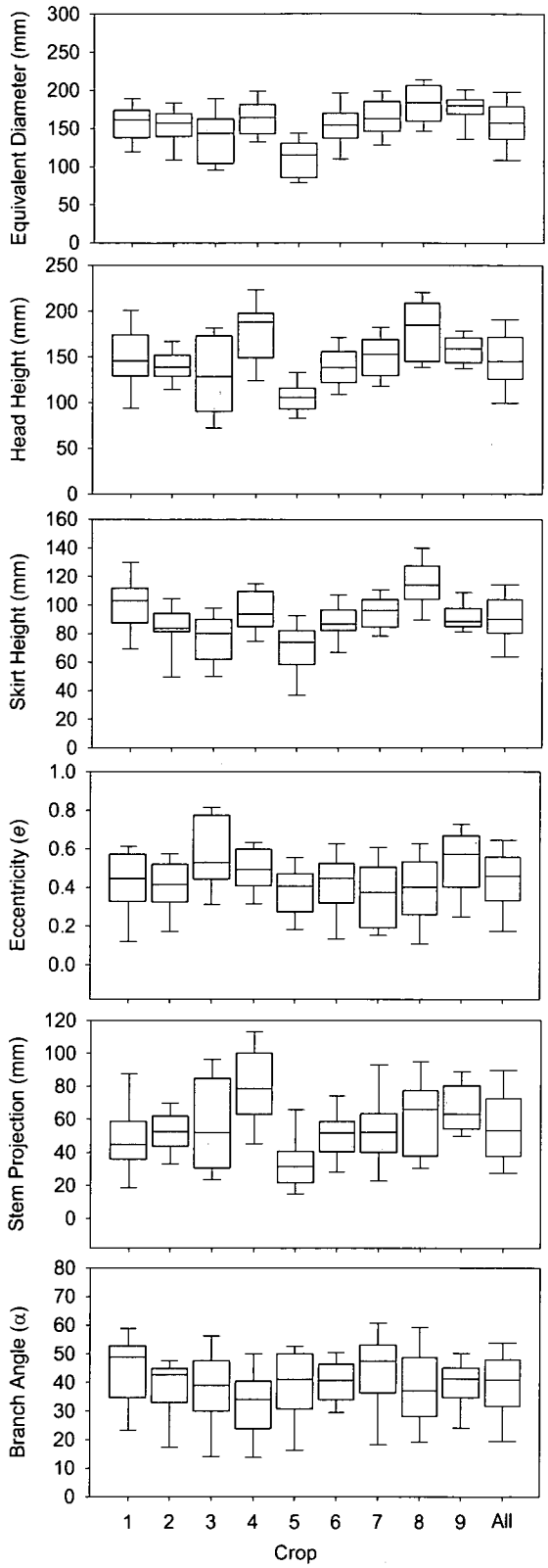


Figure 2. *Variation in broccoli 'Marathon' head architectural attributes across nine sites. Bars in grey are individual crops. Median values are indicated by the solid black line, box boundaries are the 25th and 75th percentiles and whiskers represent the 10th and 90th percentiles. The median value and distribution for all crops is represented by the white box whisker plot (All).*

INFLUENCE OF HEAD ARCHITECTURE ON PROCESSING EFFICIENCY

While similar in diameter, the heads of 'Shamrock' were heavier ($P \leq 0.05$) and the average shape more compact than 'Marathon' (Table 3, Figure 3). The mean height and core length of the 'Shamrock' heads was almost half of that of 'Marathon' ($P \leq 0.001$) and had 85% less stem projection ($P \leq 0.001$). 'Shamrock' also had slightly deeper skirts than 'Marathon' ($P < 0.05$). Head curvature of the two cultivars was similar and almost all heads (97% of 'Marathon' and 100% 'Shamrock') had curvature higher than that of the Decora receptacle.

Table 3. *Mean morphological characteristics of broccoli 'Marathon' and 'Shamrock' inflorescences. Statistical differences between the two cultivars for each of the traits were tested statistically using either ANOVA or Kruskal Wallace non parametric test (np). *P≤0.05, ***P≤0.001; ns = not significant.*

	Marathon		**Shamrock**		
Shape Parameter	Mean	SEM	Mean	SEM	Sig.
Head Weight (g)	478.6	17.24	556.4	30.0	*
Diameter (mm)	158.7	2.1	153.7	2.1	ns
Head Height (mm)	207.2	8.3	117.6	2.4	*** np
Skirt Height (mm)	96.3	1.7	100.0	1.8	*
Eccentricity (*e*)	0.48	0.03	0.43	0.03	ns
Stem Projection (mm)	110.9	8.0	17.6	2.7	*** np
Core Length (mm)	104.8	2.6	50.0	2.6	***

The core component from 'Marathon' heads was on average longer and heavier than those of 'Shamrock' whilst the latter variety produced greater total floret weights (Table 4). Consequently the core:floret ratio was superior in 'Shamrock', producing 5 grams of floret for every 1 gram of core tissue. Conversely, 'Marathon' produced 2 gram of floret for every 1g of stem material. Segments were rated as over sized, on target or under sized based on factory specifications for floret diameter and length. While having a poorer core:floret ratio, the 'Marathon' head shape produced 4.1% more florets that were in the target range for both length and diameter specifications.

Table 4. *Segmentation of core and floret segments for broccoli 'Marathon' (n =27) and 'Shamrock' (n = 22). Mann - Whitney U non parametric test (np) was used to assess treatments effects. *P≤0.05, **P≤0.05***P≤0.001; ns = not significant.*

	Marathon		Shamrock		
	Mean	SEM	Mean	SEM	Sig.
Core (g) F^{wt}	157.01	7.13	94.71	7.23	*** np
Total Floret (g) F^{wt}	321.62	11.36	461.68	25.09	*** np
Core: Floret	0.49	0.02	0.21	0.01	*** np
Target sized florets (%)	42.7		38.6		***np

Although both varieties produced similar proportions of on-target segments, 43.4% and 29.3% of the segments were oversized in at least one dimension for 'Shamrock' and 'Marathon' respectively (Figure 4). In a factory environment, these segments would require further downstream dicing to meet target specifications. Almost all oversized segments in both varieties exceeded the maximum diameter specification, with 12% by number exceeding both diameter and length specifications in 'Shamrock'. The proportion of 'Marathon' segments oversized in both dimension was almost half that of 'Shamrock' at 5.2 %. 'Marathon' produced 27.7% segments by number that were undersized in at least one or both dimensions whilst this figure was comparatively lower for 'Shamrock' at 16.8%.

Figure 3. *Side elevation of a typical 'Marathon' and 'Shamrock' inflorescence prior to, and after a radial longitudinal cut to reveal the internal branch structure. Lower overall height, shorter core length and less stem projection make 'Shamrock' more compact.*

INFLUENCE OF HEAD ARCHITECTURE ON SEGMENTATION

While it is obvious that larger, heavier heads are going to produce greater portions of both floret and stem tissue, the rate at which these accumulated as head size increased in both 'Marathon' and 'Shamrock' was different (Table 5). In both varieties floret weight increased at a faster rate than core fresh weight, and the rate of floret increase in 'Shamrock' was 25% greater than that of 'Marathon'. The rate of increase in core fresh weight with head weight in 'Marathon' was twice that of 'Shamrock'. Thus an increase in head weight for 'Shamrock' will produce proportionally more floret and less core than 'Marathon'. An increase in head weight was also moderately associated with a slight reduction in on-target segments in 'Shamrock', and this relationship was much weaker in 'Marathon'.

Similarly, as head diameter increased floret fresh weight also increased at a faster rate than the core fresh weight in both varieties (Table 5). The rate of accumulation of floret tissue in 'Shamrock' with diameter was almost twice that of 'Marathon'. There was also a weak tendency for increasing diameter to produce less target sized and more undersized segments. There was also a weak association for an increase in diameter in 'Shamrock' to produce less on target segments and more segments that required re-dicing.

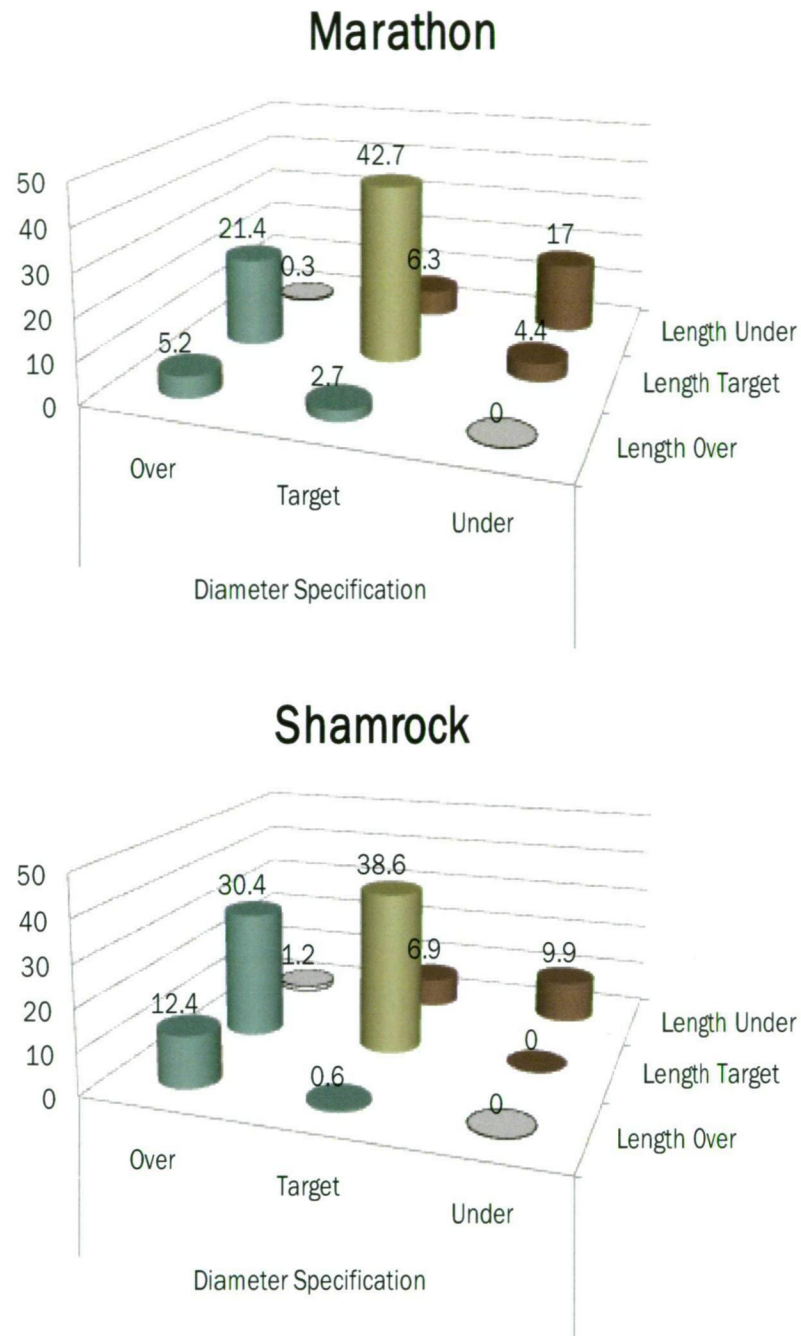


Figure 4. *Proportion of segments categorized as over sized, on target or undersized, expressed as a percent of total number of florets for each variety. Ratings were based on factory specifications for floret length and diameter.*

Increases in head length, stem projection and core length all produced a slight increase in the core:floret ratio in both 'Marathon' and 'Shamrock', yet this was not of much importance as the rate of change with each parameter was small. An increase in head length was however strongly associated with an increase in core fresh weight in 'Shamrock', while a similarly weak association could be seen for stem projection.

Skirt height was weakly correlated with an increase in core weight, floret weight, and the core:floret ratio in 'Marathon', and with an increase in floret fresh weight in 'Shamrock'. Skirt height was also weakly associated with a decrease in target sized segments and a corresponding increase in undersized segment in 'Marathon'.

Curvature did not influence the processing outcomes with the exception of a weak tendency for flatter heads in 'Marathon' to produce more segments that require re-dicing. In theory a shape with a higher degree of curvature would be expected to produce a greater tendency to tip, however, despite the two head shapes having similar degrees of curvature, Shamrock had a greater tendency to tip ($P < 0.05$) during the decoring process.

Blade travel through the inflorescence of both cultivars was predominantly through the second order branches with comparatively little segmentation occurring at higher order branch levels (Table 6). The position of the terminal cut on the main stem was at a higher II order branch position for 'Marathon' than 'Shamrock'. The variation in the position of the terminal II order cut for 'Shamrock' (CV = 34%) was also twice that of 'Marathon' (CV = 15%). Due to the higher terminal cut 'Marathon' produced almost twice as many florets less than half the weight of 'Shamrock' (Table 7). The CV for floret number (26%) and mean floret fresh weight (21%) was also less than that for floret number (39%) and mean floret fresh weight (36%) in 'Shamrock'.

Table 5. *Results of linear regression between the head shape descriptors and measures of processing efficiency. Significance is indicated by *P 0.05, **P ≤ 0.01, **P ≤ 0.001.*

		Marathon		Shamrock	
Independent	**Dependent**	**R^2**	**Slope**	**R^2**	**Slope**
Head Weight	Core F^{wt}	**0.793*** **	**0.368**	**0.553*** **	**0.179**
	Floret F^{wt}	**0.918*** **	**0.632**	**0.963*** **	**0.821**
	Core:Floret	0.048^{ns}	0.000	0.014^{ns}	0.000
	Undersize	**0.155***	0.066	0.099^{ns}	0.034
	Target	**0.276****	**-0.090**	**0.412*** **	**-0.101**
	Re-dice	0.035^{ns}	0.025	0.196*	0.067
Diameter	Core F^{wt}	**0.555*** **	**2.485**	**0.540*** **	**2.463**
	Floret F^{wt}	**0.853*** **	**4.907**	**0.572*** **	**8.795**
	Core:Floret	0.001^{ns}	0.000	0.004^{ns}	0.000
	Undersize	**0.185***	0.579	0.013^{ns}	0.171
	Target	**0.319****	-0.782	**0.313****	-1.228
	Re-dice	0.037^{ns}	0.203	**0.249****	**1.056**
Head Height	Core F^{wt}	0.103^{ns}	0.275	**0.684*** **	**2.446**
	Floret F^{wt}	0.003^{ns}	0.070	0.071^{ns}	2.739
	Core:Floret	**0.494*** **	**0.001**	**0.481*** **	**0.004**
	Undersize	0.024^{ns}	0.054	0.104^{ns}	0.425
	Target	0.014^{ns}	-0.042	0.026^{ns}	-0.312
	Re-dice	0.026^{ns}	-0.044	0.004^{ns}	-0.112
Skirt Height	Core F^{wt}	**0.172***	1.756	0.340^{ns}	1.340
	Floret F^{wt}	**0.188***	2.926	0.212*	6.287
	Core:Floret	**0.147***	0.003	0.006^{ns}	0.001
	Undersize	**0.266****	0.880	0.015^{ns}	0.213
	Target	0.204*	-0.793	0.068^{ns}	-0.672
	Re-dice	0.004^{ns}	-0.087	0.034^{ns}	0.460
Eccentricity	Core F^{wt}	0.000^{ns}	3.261	0.006^{ns}	16.168
	Floret F^{wt}	0.013^{ns}	-33.791	0.001^{ns}	23.593
	Core:Floret	0.006^{ns}	0.032	0.002^{ns}	0.017
	Undersize	0.078^{ns}	-21.215	0.026^{ns}	-2.517
	Target	0.001^{ns}	-1.929	0.002^{ns}	6.112
	Re-dice	0.150*	23.144	0.001^{ns}	-3.594
Stem Projection	Core F^{wt}	0.061^{ns}	0.221	**0.275****	1.421
	Floret F^{wt}	0.001^{ns}	-0.054	0.005^{ns}	-0.678
	Core:Floret	**0.411*** **	0.001	**0.339****	0.003
	Undersize	0.023^{ns}	0.055	0.045^{ns}	0.256
	Target	0.001^{ns}	-0.011	0.032^{ns}	0.056
	Re-dice	0.024^{ns}	-0.044	0.033^{ns}	-0.312
Core Length	Core F^{wt}				
	Floret F^{wt}	**0.168***	1.800	0.071^{ns}	2.547
	Core:Floret	**0.601*** **	**0.005**	**0.462*** **	**0.003**
	Undersize	0.059^{ns}	0.271	0.164	0.498
	Target	0.083^{ns}	-0.329	0.031^{ns}	-0.319
	Re-dice	0.004^{ns}	0.058	0.011^{ns}	-0.179

Table 6. *Blade travel by cut location (branch order) and residual florets. Clean cuts were recorded as branch stumps with no residual florets. Residual florets recorded as the lowest order branch in the residue. The terminal cut is the highest cut II order branch +1.*

	Variety			
	Marathon (*n* = 27)		**Shamrock (*n* = 22)**	
	Mean	SEM	Mean	SEM
# Clean II order cuts	10.04	0.31	6.30	0.51
# Clean III order cuts	1.74	0.47	0.30	0.21
# Residual III order florets	0.59	0.16	0.35	0.15
# Clean IV order cuts	0.11	0.11	0.00	0.00
# Residual IV order florets	0.07	0.07	0.00	0.00
Terminal cut	11.04	0.31	7.30	0.51

Table 7. *Individual floret weight, number and dimensions for 'Marathon' (n = 27) and 'Shamrock' (n = 22).*

	Marathon		**Shamrock**		
	Mean	SEM	Mean	SEM	Sig.
Mean Floret (g) Fw	24.79	0.99	69.37	5.37	*** np
Floret Length (mm)	53.63	0.97	55.03	1.15	ns
Floret Diameter (mm)	54.41	1.07	69.06	2.00	*** np
# Floret Segments head^{-1}	13.52	0.69	7.32	0.61	***

DISCUSSION

The shape of the broccoli inflorescence is determined by the production of branch primordia in a spiral phyllotaxis, the location of floral parts, and the time at which they appear (Benlloch et al., 2007). Some aspects, such as the production of branch primordia in a spiral phyllotaxis, are likely to be tightly controlled by plant genetics (Hardwick, 1984) and the results of this investigation show that each variety did indeed have a distinct shape, this difference presumably associated with gene expression. Other aspects that determine branch patterning such as the

iteration interval and the relative rate of primordial production (Kieffer et al., 1998), while also likely to be determined by genetics, might also be influenced by environmental factors. The results of this study show that head shape was variable within the variety 'Marathon', particularly within a site, indicating that there is some influence of environmental factors on the expression of head shape.

THE INFLUENCE OF HEAD SHAPE ON PROCESSING EFFICIENCY

The differences in shape and branching architecture of 'Marathon' and 'Shamrock' inflorescences influenced processing efficiency through net floret yield, the core:floret ratio and the proportion of segments that fell within the target size class. The compact head shape of Shamrock provided it with superior net yield and core:floret ratio over the lanky shape of 'Marathon'. The superior core: floret ratio conferred by its shorter core and the higher increase in floret fresh weight per unit increase in head size (diameter / fresh weight), indicates that greater gains can be made from increases in gross yield with compact head shapes. The limited core length in 'Shamrock' would also provide an opportunity to manipulate the quantity of core harvested to meet factory requirements. When the requirements for core are low, heads may be cut to the lowest branch insertion point, providing minimal quantities of core, while in seasons with greater core requirements additional stem could be cut. In addition to presenting a limited opportunity to manipulate the core length, the rate of increase in core fresh weight with head size in 'Marathon' was twice that of 'Shamrock'. Thus for this taller head shape, an increase in gross yield will also be associated with an increase in the less desirable core.

Core length, and hence weight, is determined by the insertion point of the lowest branch in the inflorescence. Thus the amount of core harvested is essentially a function of branch angle and head diameter, as an increase in diameter at a constant branch angle necessarily dictates an increase in elongation of the main axis and II+ order branches, particularly those held in lower positions. Assuming that branch angle is largely determined during primordial development, it then follows that head length must in general increase with diameter. This assumption is to some extent mitigated by the orientation of the higher order branches which

can extend in a horizontal plane, but would hold true for the overall structure. Heads with a less acute branch angle will produce shorter I order axes. As the heads from both samples were on average the same diameter, the differences in height of the heads can be attributed to a less acute branch angle in 'Shamrock' providing this variety with a shorter main axis and low stem projection. Thus the superior core:floret ratio associated with the compact shape of 'Shamrock' was probably, predominantly endowed by the branch angle derived during primordial development of the inflorescence.

Thus there may be some potential to use plant husbandry to manipulate head shape and improve the core to floret ratio. Increased planting density for example, results in reduced light quantity and quality, which in turn produces greater inter-nodal elongation and more acute leaf angles (Smith, 1997, Whitelam and Johnson, 1982). Assuming a similar response in branch angle for broccoli, a lower planting density or a less dense leaf canopy may produce heads with a lower branch angle. This in turn would lead to a decrease in core length and possibly improve the core to floret ratio, particularly in 'Marathon'. Therefore changes made in agronomic practices which alter the growing environment may influence some of the physiological components that determine other architectural determinants of the inflorescence.

While the net floret yield and core:floret ratio of 'Shamrock' exceeds that of 'Marathon', this latter variety was 18% more efficient to process. The greater head length of 'Marathon' is possibly responsible for this variety's improved processing efficiency because of its influence on blade trajectory. For heads of equal diameter, the longer core length (I order axis) of 'Marathon' would require greater elongation of those branches held in lower positions and might also increase inter-nodal distances between II order branches. This in turn would produce a comparatively more open branch structure on a longer axis that increases the likelihood of blade travel through more II order branches before the terminal cut on the main stem is made. The increased number of II order branch cuts lead to the derivation of a greater number of florets of smaller diameter and less weight as reflected in the proportion of segments in the target and undersize categories for this variety. Thus the taller and more open branch structure (Figure 3) of

'Marathon' led to the production of a comparatively larger number of smaller florets.

In contrast, the compact nature of the 'Shamrock' inflorescence led to the production of lower number of heavier branch segments of larger diameter. The greater size of these segments was because the majority were derived from low positioned II order branches which generally carry larger floret structures. The low order terminal cuts would also have produced larger terminal segments, this also contributing to the higher mean floret weight observed in 'Shamrock'. The greater weight of these segments was a function of increased floret diameter, as the mean floret length was not different between the two varieties.

Close to all of the heads had a higher degree of curvature than the apparatus used to hold the head in place during segmentation. Theoretically, the greater this departure in curvature, the more likely it is that the inverted inflorescence will rock out of alignment when the conveyor stops if it is not fixed properly by the steel spikes inserted into its dorsal surface. While 'Marathon' had a similar degree of curvature and presumably a higher centre of gravity attributed by greater stem projection, the number of heads tipped out of alignment was greater for 'Shamrock'. One explanation for this is that the heads of 'Shamrock' may not be fixed by the steel spikes as solidly as 'Marathon', possibly due to difference in 'tissue' strength between varieties. In a factory setting, the increased tendency for rocking in 'Shamrock' would have created a reduction in processing efficiency for this variety.

In summary, there are distinct advantages and disadvantages between the two head shapes. The more compact shape of 'Shamrock' provided a greater net floret yield and an opportunity to manipulate the core:floret ratio. The shape of the 'Shamrock' inflorescence will also provide greater gains in net floret yield as gross yield increases with diameter. The advantage of this heads architecture is however off set by the production of more oversized florets that require additional processing. As the same machine setting was used for both varieties in this study, it may be that modifying the blade size and trajectory to this head form may improve the segmentation for 'Shamrock'. 'Marathon' provided improved

processing efficiencies, nevertheless, this head shape provided limited opportunity for controlling the quantity of stem material harvested and thus the core:floret ratio.

THE INFLUENCE OF SITE ON VARIATION IN HEAD SHAPE

There was a range of variation in the medians between sites for each of the descriptors used to measure the effect of site on the 'Marathon' head shape, this variability being smallest for branch angle and eccentricity. Although the differences in these medians are not representative of the whole crop and cannot be attributed as such, the range of responses does indicate that the environment within each plot had some influence on head shape. Even so, most variation was attributed to within site variation. This would seem to indicate that while site may influence shape, plant genetics may make the greatest contribution to the variability observed in each trait, and that the gene expression determining phenotype is variable.

When considering between site variation across attributes, higher variation was evident for head fresh weight, diameter, height, skirt height and stem projection although this might simply be a product of differences in crop development. While harvesting at the second commercial harvest was intended to ensure an even spread of maturity, an unintended consequence of this was that some plots had not reached harvest maturity at this time. This arose because while the majority of the crop was ready for harvest, sections of the field, on occasion including the sample plot, were not as advanced. As head diameter, weight, height, skirt length and stem projection were all strongly correlated, the increase in between-crop variation in these shape descriptors could simply reflect differences in crop development between sites as entailed by the different harvest times.

This highlights the importance of harvest timing, as this influences both head diameter and length, and subsequent to this, processing efficiency. Delaying harvest to achieve the maximum possible diameter (determined by quality parameters) will not only increase net floret yield, but also improve processing

efficiency as head length will increase with diameter. This associated increase in head length will lead to the production of more florets of smaller dimensions, increasing the proportion of product within target specifications as already demonstrated.

This study has provided some evidence that both site differences and the architecture of the two heads shapes as determined by genotype influence the key parameters used to measure processing outcomes. There is significant potential for processors of frozen broccoli products to use the different head morphologies conferred by genotype and agronomic practices to improve net yield. This study provides a foundation from which further research into plant varieties and agronomic practices can be used to manage the core:floret ratio and improve processing efficiency by manipulating the size distribution of the floret segments produced.

CHAPTER 4

INFLUENCES ON INFLORESCENCE HARVEST MATURITY

INTRODUCTION

In Australia, broccoli varieties produce a main terminal inflorescence, and it is this component that is harvested. The harvest phase itself is a resource intensive exercise and its organisation and execution requires significant logistical and labour inputs. Each harvest event requires the organisation of teams of 10 or more people, bins for storage, tractors to collect and load the produce, and trucks for transport to the factory. While some of these costs are fixed, the costs of other variable components generally increase with the number of times that a crop must be visited in order to harvest all plants. As individual broccoli heads reach harvest maturity at different times, harvesting these heads at an appropriate age that maximises both yield and quality currently necessitates up to 6 cuts or visits by harvest teams to the crop. The number of cuts required is mostly due to variation in plant development although a smaller component also arises from differences in the perception of maturity by field staff. The number of harvest events required means that harvesting, particularly the labour component, is a significant factor in the cost of raw produce. Providing a solution to reduce the number of harvest cuts will help reduce the unit cost of raw produce and improve the scheduling of supply to the factory. In an environment where a considerable disparity between labour costs allows other countries to provide product of the same quality at cheaper prices, a reduction in labour costs during harvest is essential to make the local Tasmanian industry competitive.

Very little is understood about how or when variability in harvest maturity is introduced into a broccoli crop, although there is some indication that variability in floral initiation during early crop establishment may be of importance (Wurr et al., 1990, Booij, 1990a). In cauliflower, most variation in curd initiation is related to

variation in the end of the juvenile phase and the mean maximum daily temperature during the adult vegetative phase and curd initiation. Some of the variation in harvest duration (55%) is explained by variation in the length of the curd initiation phase and temperature during curd development (Booij, 1990a). In broccoli ('Corvet'), at least one study has reported that the variability in the time from transplant to head initiation (meristem diameter = 0.6mm) is less than variability in the time from head initiation to harvest, when using the range of means across years and planting dates for comparison (Wurr et al., 1991).

Improving on the current understanding of the stage of crop development at which variation is introduced and of the mechanisms involved would allow for a reduced number of harvests and a decrease in the associated costs. Crop uniformity is also a prerequisite for once over harvesting which in turn would permit the development of mechanical harvesting, further reducing the costs associated with harvest.

Broccoli crops in Tasmania are transplanted at the 3-4 leaf stage when plants are 4-5 weeks old. In the nursery the seedlings are raised outside at ambient temperatures while nutrition, root growth and soil moisture are tightly controlled. The relatively low temperature experienced during this time (mean min. 10 °C) is sufficient to vernalise the seedlings. Hence the presence or absence of a juvenile phase is of importance, as nursery management practices may influence the seedlings transition from a juvenile to adult vegetative phase. The transplanting process itself can be an arduous experience for seedlings as their new environment is much more variable with respect to soil moisture status, soil structure and nutrition. Due to the logistical limitations of transplanting large areas (e.g. irrigation often cannot start until planting is completed), the plants often become drought stressed before receiving their first irrigation. These events provide a significant challenge to successful seedling establishment, and may influence the progress of individual plants towards flowering. After transplanting the seedlings are regularly watered, and side dressed with fertiliser on two occasions. At approximately 8 weeks after transplanting the inflorescence becomes visible after having reached 10-15 mm in size. Inflorescence development to a size approximating 150mm occurs over the next 4 weeks.

Thus the stressors, commercial management practices and environmental conditions experienced during transplanting and crop growth may influence the seedlings response to floral evocation. The delayed onset of floral initiation could in turn lead to variation in floral initiation between plants. It is in this context that the juvenile phase, the requirements for floral evocation and the influence of cultural and environmental factors are of importance as possible contributors to the variability in harvest maturity in commercial broccoli crops.

GERMINATION AND THE JUVENILE PHASE

As nursery conditions provide an environment that may be conducive to floral evocation if the plants are indeed competent at this stage, knowing the age or stage of development at which broccoli seedlings become available to receive the flowering stimuli would allow for the development of management practices that maximise uniform floral initiation.

Most plant species pass through a phase in which seedlings and young plants are not responsive to floral evocation via internal or external stimuli. Although not always present in annual plants (Wiebe, 1990), this phase is referred to as the juvenile phase and may be accompanied by morphological differences with respect to older vegetative plants (Taiz and Zeiger, 2002). Friend (1985) notes that a juvenile stage has been reported for cauliflower (Kato, 1964, Sadik, 1967, Wurr et al., 1981) and broccoli (Fontes et al., 1967, Fontes and Ozbun, 1972) in addition to other cultivated *Brassica* species. The juvenility reported by Fontes *et. al.* (1967) was for sprouting broccoli cultivars which were not responsive to chilling at 4.4°C until 4-5 weeks after germination. Broccoli plants are also reported to be available for induction when plants have four or more leaves > 2cm (Wiebe, 1990).

There are discrepancies in the literature, and the argument for the absence of a juvenile phase in this annual crop is supported by reports that some broccoli cultivars produce a vernalisation response to the chilling of imbibed seeds during germination (Miller, 1988, Fujime and Hirose, 1979). However, in other similar studies no response has been observed (Gauss and Taylor, 1969). Wien and Wurr

(1997) suggest the apparent discrepancy may be due to differences between cultivars or that sensitivity to vernalisation may decrease after germination and then increase again at the end of a juvenile phase. The optimal temperature for 'after effect' seed vernalisation of *Brassica* species is purported as 5°C (Friend, 1985).

The absence of a juvenile phase has also been postulated from the modeling of crop ontogeny in broccoli. A number of models have been developed to predict the time to harvest maturity of headed and sprouting (calabrese) phenotypes (Mourao and Brito, 2000, Grevsen, 2000, Grevsen and Olesen, 1999, Marshall and Thompson, 1987, Tan et al., 1999a, Tan et al., 2000a, Wurr, 1992, Wurr et al., 1995, Grevsen, 1998). Two of these studies discount a juvenile phase (Wurr et al., 1995, Grevsen and Olesen, 1999) however in both of these instances the data used to validate the models was recorded from transplanting onwards. Wurr *et al.* (1995) note that the presence of the parameter *a* in their model, the diameter at which the expansion rate becomes exponential, is an indication of a juvenile phase (*i.e.* exponential models have a lag phase). Nevertheless, if present, the authors concluded it had already passed by transplanting.

FLORAL EVOCATION

Both differences in the time at which plants become competent and variability in the flowering stimuli received by individual crop plants spread across a heterogeneous area, may lead to differences in the timing of floral induction between plants within a crop. The events that effect the transition of the apical meristem from a vegetative to a floral constitution are referred to as floral evocation. This phase change can be regulated internally (autonomous), may be facilitated by external factors (facultative), or have a strict requirement for external factors (obligate). Vegetative plants that are able to respond to evocative stimuli are referred to as competent. After a sufficient period of stimulus, competent apical meristems will continue to develop as flowers even if the initial stimulus is removed, although other signaling agents such as hormones may still be required for flowering to continue (Taiz and Zeiger, 2002).

Floral evocation is principally driven by vernalisation and photoperiod, these being referred to as primary factors. The effect of these two primary factors can in turn be modified by secondary factors such as ambient temperature, irradiance and water availability, or tertiary factors such as mineral availability, light quality and neighbouring vegetation. The influence of any of these factors on evocation is perceived by the meristems, shoot, roots, leaves or any combination of these four. Thus while primary factors normally drive the flowering process, their influence may become redundant, suppressed or overridden by secondary or tertiary factors (Bernier and Perilleux, 2005).

Friend (1985) in his review of flowering of *Brassica's* notes that while commonly facultative long day plants, the vernalisation and photoperiod requirements for this genus may be substantially different between species. Broccoli is recorded as having a preferential (facultative) response to a low temperature chilling response that is maintained even if non-inductive conditions occur later ('After Effect Vernalisation').

VERNALISATION

The range of temperatures for which floral induction is reported in broccoli is broad, spanning -2.8°C to 30°C (Table 1). Under constant temperature conditions, vernalisation proceeds fastest at 15 - 16°C (Wurr et al., 1995, Grevsen, 2000, Fellows et al., 1997, Fujime and Hirose, 1980) while 'After effect' vernalisation is known to be effective in broccoli at 5°C (Fontes et al., 1967, Fontes and Ozbun, 1972). In one of the few studies undertaken using diurnal temperatures under controlled conditions, the maximum rate of floral initiation and development in broccoli occurred at 15/10°C and 15/15°C day night temperatures (8 / 16 hr) (Fujime and Hirose, 1980). Other studies have found no vernalisation requirement for broccoli (Mourao and Brito, 2000) with flowering still proceeding up to a constant 29.4 °C (Gauss and Taylor, 1969).

Evidence provided from the literature supports the classification of broccoli as a facultative plant with a preference for after effect vernalisation, although some

studies have concluded that broccoli has no chilling requirement at all (Gauss and Taylor, 1969). There is certainly a temperature above which flowering does not occur as evidenced by the maxima provided in Table 1, indicating that the response to floral stimuli is not autonomous. It remains however that some cultivars held at comparatively high constant temperatures will still flower, despite not having been exposed to "true" chilling temperatures such as 5 °C reported by some studies (Fontes et al., 1967, Wiebe, 1990, Fontes and Ozbun, 1972). The wide range of temperatures at which vernalisation occurs suggests a facultative rather than obligate requirement for vernalisation. Wiebe (1975) argues that while Gauss and Taylor (1969) found no vernalisation requirement for cultivar 'Coastal' with respect to time to floral initiation, the increase in leaf number associated with an increase in temperature in Wiebes' experiments indicates a facultative vernalisation response for this cultivar.

Vernalisation in broccoli has been linked to a rapid increase in the size of the vegetative apical meristem, with flowering typically being initiated at a meristem diameter ranging from 400-600 µm. On average, reproductive transition occurs at an apex diameter of approximately 500 µm. (Wurr et al., 1995, Kieffer et al., 1998, Tan et al., 1998). Shoot apical meristem (SAM) expansion occurred under constant temperatures of 7.3°C to 19.2°C for cultivar 'Shogun', with the fastest expansion rates occurring at 14.8°C and 15.6°C (Wurr et al., 1995). While there is no distinct weight or size at which floral transition occurs, plants appear to attain a minimum leaf number, stem diameter and weight before the vegetative apex reaches a sufficient size for this process to begin. Plant size, weight and leaf number at floral transition will therefore vary in response the rate of apex expansion, as determined by environmental conditions (Wurr et al., 1995).

Given that there are temperatures above which flowering does not occur, it can be concluded that broccoli is strictly obligate. Nevertheless, due to the unconventionally high temperatures at which vernalisation may occur, particularly in the field, broccoli effectively functions as a facultative plant. Cool overnight temperatures are likely to be sufficient to induce vernalisation, as evidenced by an

increase in the size of the vegetative meristem (Wurr et al., 1995) in response to evocation. The subsequent rate at which the apex diameter increases in size as the plant progresses toward flowering is likely to be driven by ambient day time temperatures. The closer this value is to 15-16°C, the faster the apex expansion rate and the earlier inflorescence production will occur.

Table 1. *Reported cardinal vernalisation temperatures for inflorescence initiation in broccoli.* [SP] *= sprouting phenotype* [HP] *= headed phenotype*

Investigator	Cultivar(s)	Cardinal Temperatures			Period of exposure	Data Source
		Base	Optimum	Maximum		
(Fellows et al., 1997)	Shogun [HP] Corvet[HP]	0°C	15°C	30°C		Model supported by field data.
(Fontes et al., 1967)	Waltham 29 [HP] Green Mountain[HP]		4.4°C after effect	24-27°C	3-6 wks	Growth chamber data.
(Fontes and Ozbun, 1972)	Waltham 29 [HP]		5°C after effect		3 wks	Growth chamber data.
(Fujime and Hirose, 1980)	Wase-midori[HP]		15/15°C 15/10°C	25/25°C	5 wks	Growth chamber data.
(Gauss and Taylor, 1969)	Coastal [SP]	No cold requirement detected.				Growth chamber data.
(Grevsen, 2000)	Caravel[HP] Shogun [HP] Emperor[HP]	2.9°C	16.3°C	29.7°C		Model (r^2=0.58) supported by field data.
(Mourao and Brito, 2000)	Compacta[HP] Comanche[HP] GreenValiant [HP] Marathon [HP]	No cold requirement detected.				Field data.
(Tan et al., 2000b)	Fiesta[HP] Greenbelt [HP] Marathon [HP]	0°C	20°C			Thermal time model supported by field data.
(Wiebe, 1975)	Coastal [SP] Gem [SP]		17°C (my interpretation)	>27°C		
(Wiebe, 1990)		0°C	5°C	20°C	2-4 wks	Review
(Wurr et al., 1995)	Shogun [HP] Corvet[HP]	-2.8°C	15.8°C	23.6°C		Thermal time model supported by field data.

PHOTOPERIODISM

The reported photoperiodic requirement for *Brassica spp.* is again quite varied, mirroring that of vernalisation. In general plants of this genus are facultative long day plants, with some plants being day neutral if vernalisation has occurred (Friend, 1985). No obligate requirement for day length has been demonstrated in broccoli however there is some evidence to suggest that some cultivars have a facultative requirement, probably redundant to vernalisation. Cultivar 'Coastal' putatively has a facultative photoperiod requirement (Gauss and Taylor, 1969). When exposed to 8,16 and 24hr day lengths, the number of days to buttoning in this green sprouting broccoli generally decreased with increasing day length at both 13°C and 29°C, with the exception of 29°C/24hr combination under which the time to flowering increased. Fujime et al. (1988) screened 13 cultivars for photo-thermal induction observing that at 20°C, early and intermediate lines formed inflorescences under long days but not short. At 17°C, presumably at which all cultivars formed heads, some plants under long day regimes had fewer leaves and flowered up to one week earlier. The investigators concluded that temperature had a greater influence on spear initiation when compared to day length. Tan et al. (2000b) also concluded that cultivars 'Fiesta', 'Greenbelt' and 'Marathon' showed a slight response (1 day earlier EFI) to an increase in day length from 11 or 12 hours to 16 hours when grown in South East Queensland, Australia.

CULTURAL AND ENVIRONMENTAL INFLUENCES ON THE TIMING OF INFLORESCENCE MATURITY

The agronomic management of broccoli crops is comprised of a series of events that involve the manipulation of secondary and tertiary factors that may influence floral evocation. Seedlings are germinated in an artificial environment with ambient temperatures, restricted root volume and tightly controlled nutritional and moisture regimes. At transplanting these seedlings are then placed in the much more variable soil environment of the paddock, bringing about a period of rapid change during which the plant must adapt to its new environment. During

the period from transplant to floral initiation, agronomic management, a heterogeneous soil environment, increasing interplant competition and environmental factors will influence water availability, ambient temperature, the quantity and quality of irradiance and mineral availability, all factors which may impinge on competency and floral evocation. Thus both cultural and environmental influences may alter the timing and uniformity of inflorescence maturity.

DENSITY AND TIME OF TRANSPLANTING

Rising plant population densities increase crop yields until an asymptote is reached at 20 plants m^{-2} (Chung, 1982, Salter et al., 1984). As the planting density increases, head diameter and weight decline while also becoming more variable in nature (Cutcliffe, 1971, Wurr et al., 1992, Chung, 1982, Salter et al., 1984, Damato, 2000). Although head size becomes more variable at higher densities, a similar variability in the timing of inflorescence initiation has not been observed (Wurr et al., 1992). Higher planting densities can however delay harvest maturity in some varieties (Cutcliffe, 1971) although this is not a consistent response and the time to maturity is often shortened (Wurr et al., 1992, Chung, 1982, Thompson and Taylor, 1976).

The time of planting appears to have a greater influence on the timing and variability of harvest maturity, particularly if combined with high plant densities. In both broccoli and cauliflower, late season plantings in concert with higher plant densities introduce greater plant-to-plant variability in harvest maturity when compared to early season crops (Chung and Strickland, 1986, Salter et al., 1984, Sorensen and Grevsen, 1994). The time to floral initiation also increases with later autumn plantings (Tan et al., 2000b). These factors, in addition to slower relative growth at lower temperatures experienced by later plantings, possibly magnify any variation in the timing of floral initiation between plants.

TRANSPLANTING

The developmental stage at which seedlings are transplanted, and the way in which they are transplanted, has an effect on yield, time to harvest and the uniformity of harvest maturity of broccoli. Seedlings may be transplanted either bare rooted or from plugs grown in acrylic trays. Transplanting from tray cells improves harvest uniformity, however there are reports that this system may reduce the yield in some cultivars (Giovanni and Vincenzo, 1988).

Cell dimensions and volume influence plant size and morphology with plants generally becoming heavier and carrying more leaves as cell width increases, and becoming taller with cell depth (Dufault and Waters, 1985). Despite increasing stature with cell volume, this does not generally appear to increase yield in broccoli (Marr, 1985, Dufault and Waters, 1985, Terry Jones et al., 1991) although small cell sizes (e.g. 4.78 cm^3) have reduced yield in some studies (Damato et al., 1994). Likewise, the influence of cell size on harvest maturity has been varied, with some workers reporting a delay with small cell sizes (Marr, 1985, Terry Jones et al., 1991) and others no effect (Dufault and Waters, 1985). Cell shape also influences seedling development but is unlikely to affect harvest uniformity (Damato and Trotta, 2000).

Growing transplants to a size beyond which the plants growth rate becomes restrained at a particular cell volume is likely to reduce yield (Wien, 1997) and can influence harvest uniformity. Damato et al. (1994, 2000) have shown that for broccoli holding transplants 7 and 14 days beyond 3 true leaf stage can progressively increase the time to harvest, this effect being exacerbated by smaller cell volumes. While increasing the time to harvest, holding 14 days past the 3 leaf stage improved harvest uniformity by 9 days although yield was reduced.

IRRIGATION AND NUTRITION

Both irrigation and fertilisation have been shown to influence the timing and uniformity of harvest in broccoli (Babik and Elkner, 2002, Dufault, 1988). Babik and Elkner (2002) found that under high rates of nitrogen (400-600 kg N ha^{-1}, this including residual N), the time to harvest was reduced by 7 to 15 days when

compared to lower rates of 100 – 200 kg N ha^{-1}. Increasing the rate from 100-200 kg N ha^{-1} in itself also reduced the time to harvest by 11 days when applied as a single application. The time to harvest was also reduced under irrigation when compared to dry land plots, the mean decrease when nitrogen treatments were pooled being 6 days. Similarly, in a glasshouse study, increasing N from 1.9 g to 5.6 g per pot increased plant growth rate and reduced the time to harvest (Dufault, 1988).

TEMPERATURE

Modeling and field studies have shown that the time to inflorescence initiation in broccoli is dependent on temperature (e.g. Effective Day Degrees), with suboptimal temperatures delaying this stage (Tan et al., 2000b, Diputado and Nichols, 1989, Grevsen, 1998, Mourao and Brito, 2000, Wurr et al., 1995). Optimal temperatures used in modeling the time to floral initiation and harvest maturity vary between cultivars and developmental stage and have been reported as being anywhere from 5 ^{0}C – 20 ^{0}C, while base temperatures commonly approximate 0 ^{o}C (Tan et al., 2000b, Diputado and Nichols, 1989, Grevsen, 1998, Mourao and Brito, 2000, Wurr et al., 1991). In regions where solar radiation is limited by climatic conditions, the inclusion of solar radiation in effective day degree models has improved the prediction in broccoli crop development, while in other areas, this parameter is not necessary (Wurr et al., 1991, Tan et al., 2000b, Grevsen, 1998). These studies have shown the time to floral initiation under similar environmental conditions is influenced by cultivar and that the response to temperature appears to be more stable within some cultivars than others. The effect of temperature on the time from seedling emergence to floral initiation is also much more pronounced than its influence on the time from floral initiation to harvest (Tan et al., 2000b).

CONCLUSIONS

The development of variability in harvest maturity can be attributed to a wide range of factors that interact with the floral physiology of broccoli during the various stages of crop development. Variability in harvest maturity can be introduced though impacts that prolong a juvenile phase if this exists or through secondary and tertiary factors that alter evocation during an adult vegetative phase. Further variation might be introduced or exacerbated as the heterogeneous field environment produces small differentials in growth rate and hence development between plants after floral initiation. This temporal interaction between the stages of crop development and these factors provides for a significant permutation of possible causes and times of introduction.

Given the large number of possibilities, measuring the variability in a broccoli population's progress towards harvest maturity through its various stages of development would highlight the timing and pattern with which variability is introduced. This variability could be measured using the apex diameter and the morphological scales of inflorescence development provided by Tan et al. (1998) and Kieffer et al. (1998). Identifying the stage of development during which variability is introduced, and which stage makes the most contribution, would enable future research to focus on the environmental and management factors that are most likely to be in play.

CHAPTER 5

VARIATION IN HARVEST MATURITY IS INTRODUCED DURING FLORAL INITIATION

INTRODUCTION

Countries with low input costs are able to export processed broccoli for packaging by local processors at a cost similar to that of local raw produce (Anon., 2005). The availability of cheap processed product from China has meant that the Tasmanian processing facilities are under pressure to use this source over the more expensive local raw produce. Due to the large disparity in labour costs between China and Australia, any aspect of the supply chain that involves labour is making a significant contribution to the higher cost of raw produce. The highest demands for labour are made during transplanting and harvest of broccoli.

Variability in harvest maturity has a significant impact on the costs of harvesting broccoli. In Tasmania the terminal inflorescence of broccoli, otherwise known as the head, is harvested at a physiologically immature stage that balances maximum yield with other desirable attributes such as the tight clustering of florets and acceptable floral bud size. Due to rapid development, the window during which a head is considered to be harvest mature is quite short (Chung, 1982). This limited window of opportunity is problematic as individual plants within a crop reach harvest maturity at different times. Consequently the variability in harvest maturity can result in up to 6 harvest events, or cuts, being required to pick a crop for processing.

As broccoli is harvested by hand in teams that may consist of 10 or more people, the number of cutting events required to harvest a broccoli crop becomes a significant contributor to the cost of raw produce. Apart from direct salary costs, there is also a considerable logistical component associated with this and the

transport of the produce to the factory. Thus a reduction in the number of cuts required to harvest a crop provides a sizeable opportunity to reduce the cost of raw produce. Limiting the number of harvests to one cut would also permit the introduction of once over harvesting by hand or even mechanised harvesting.

While a number of studies have considered agronomic influences on harvest uniformity in broccoli crops (Chung, 1982, Marr, 1985, Salter et al., 1984) very little is known about which points in the crops life cycle this variability is introduced, or about the developmental events that contribute most to the differences in harvest maturity between plants. There is some indication that variability in floral initiation during early crop establishment may be of importance in cauliflower (Booij, 1990b), however for broccoli greater variation has been observed in the phase from initiation to harvest (Wurr et al., 1991). This variation may be introduced gradually during the life of the crop, or alternatively, a significant proportion may be introduced at a particular stage of crop development or at a specific crop management phase. While part of the variation is likely to be genetic, other components are possibly related to environment, competition and crop husbandry.

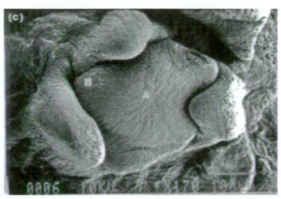
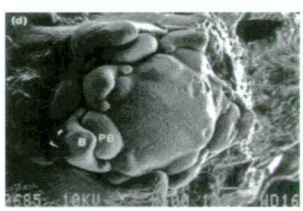
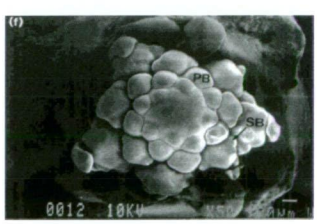
The development of a strategy to reduce variation in harvest maturity is therefore complicated by the large combination of factors that may combine to produce this phenomenon. The identification of the developmental events or stages that contribute most to crop variability would reduce the number of these permutations making it possible for further research efforts to focus on strategic points in the crops life cycle.

Identification of these key stages requires the ability to track the development of variability in harvest maturity, this in itself necessitating techniques that enable the measurement of a plants progress towards flowering. Meristem diameter and rating the morphological development of the shoot apex are two such tools. In combination, these allow variability in a plants progress towards flowering to be measured prior to inflorescence initiation, and from initiation to development of the primordial inflorescence structure. A number of studies have reported a meristem size at which floral initiation occurs, the mean diameter of the apex for

this event ranging from 390 - 513 μm (Wurr et al., 1995, Kieffer et al., 1998, Tan et al., 1998). Some of the variation in the means reported for floral initiation may be due to differences in the techniques used for measuring the meristem or in the definitions of this event (Tan et al., 1998). The exponential increase from a relatively small apex size to this diameter appears to be associated with floral evocation as broccoli held at non-inductive temperatures maintain an apex diameter as little as 0.2mm (Wurr et al., 1995). As the plants appear to transition between vegetative and reproductive states within a relatively consistent meristem diameter range, variability in apex size should reflect the associated variance in a plants progress towards flowering. Once floral identity is evident, in addition to apex diameter, scales that rate morphological development (Kieffer et al., 1998, Tan et al., 1998) of the inflorescence apex can be used to further monitor a plants progress towards maturity. The various stages and their descriptions for two of these scales have been combined below in Table 1.

The objective of this study was to identify the timing and patterning of variation in broccoli's progress towards flowering using both apex diameter and the morphological stages of development. Identifying the pattern in which variation is introduced will allow further research to target these stages of crop development, providing potential for the introduction of agronomic measures that minimise non genetic variation in harvest maturity.

Table 1. *Development of the apical meristem at different stages of transition from vegetative to inflorescence(modified after Tan et al. (2000b) and Kieffer et al. (1998); all images sourced from Tan et al. (1998).*

Stage
 **1 Vegetative** The apical meristem is small and pointed, and surrounded by developing leaf primordia. Once the evocation of flowering has occurred, the meristem rapidly increases in diameter. L = *leaf primordium*; A = *meristem proper*
2 Transition As the meristem approaches 500 µm in diameter transition to flowering occurs (Wurr et al., 1995). Just before floral initiation, the dome shaped apical meristem flattens out becoming wider, and bract primordia are initiated. A = *meristem proper;* B = *bract primordium* 
 **3 Inflorescence Initiation** Floral initiation is evident by the development of II order floral branch primordia. No initiation of leaf primordia occurs from this point onwards. PB = *II Order primordium*; B = *bract primordium*
4 Head Forming More bract and floral branch primordia are initiated in a generative spiral arrangement, each rotation approximating 137.5°. PB = *II Order primordium*; B = *bract primordium* 
 **5 Head Thickening** Further development of numerous bract and floral primordia. The branch primordia take on meristem identity as their diameter reaches 246 ± 13 µm, III order branch primordia begin initiation on the flanks of the II order inflorescence meristems. PB = *II Order primordium*; SB = *III order primordium*
6 Head Thickening III order primordia production continues and IV and V order primordia are also produced. III order and higher inflorescence primordia were produced in Kieffer et. al. (1998) study until floral identity was assumed. The rate of III order branch production was relatively constant up to II order positions 30-33. The elongation of some of the developing branch meristems precedes the transition to the floral stage.
7 Floral Initiation The transition from inflorescence to floral meristem starts not in the oldest primordia, but in the II order primordial positions 20-30 (also Wurr et al., 1995). Floral branches elongate further and 4 sepals are initiated on the tips of the elongated floral branches. In broccoli and cauliflower, lateral growth rapidly outstrips vertical growth, contributing to the semi-spheroid shape of the inflorescence. Carr and Irish (Kieffer et al., 1998) report that morphogenesis of the floral organs is similar to that described by Smyth et. al. (1997) for *Arabidopsis thaliana*.

METHODOLOGY

CROP SAMPLING

To reflect plant development under local conditions trial sites were established in 9 commercial broccoli crops in the region surrounding Devonport, Ulverstone and Penguin, Tasmania. These crops were transplanted into ferrosol soil types over a 15 day period from the 23rd January 2006 to the 7th February 2006. Plants were arranged in two rows per 1.64m wide bed at a commercial planting density approximating 34, 000 plants ha^{-1}. At each site an area a 13.2m (8 beds) x 15 m considered to be representative of the paddock was selected for sampling. Twenty plants per site were collected at transplanting direct from the seedling trays. For each plant the widest stem width (mm), stem height (mm), the number of leaves > 2cm long, shoot dry weight (g), morphological stage of development (described below) and meristem diameter (μm) was recorded. Starting at 14 days after transplanting, a further 20 plants were harvested in a strip 2-3 plants wide across the 8 beds and assessed for the same attributes as seedlings. Plants were harvested by being pulled from the soil and with the root ball remaining intact. They were then sealed in 70 L plastic bags and transported back to the laboratory where they were stored at 3°C before being assessed. Prior to assessment, the root ball was removed by cutting at the cotyledon scar. Sampling continued every 14 days until all plants had developed an inflorescence 10-15mm in diameter (buttoning stage) after which sampling ceased until harvest. The timing of the final harvest coincided with the second cut of the commercial harvest program, as at this time the sample was considered likely to contain a representative distribution of harvest maturity.

MERISTEM DIAMETER

Prior to inflorescence initiation the meristem diameter was measured using a stereo microscope fitted with a graticule at 63x magnification, producing an accuracy of ± 8 μm. The orthogonal meristem diameter was measured as the mean of the widest point between the adaxial surface of the leaf primordia and the diameter of the meristem proper at 90° to this. From inflorescence initiation onwards the diameter of the meristem was measured as the mean orthogonal diameter between the adaxial surface of the bracts subtending the two outer most branch primordia (Wurr et al. 1991,1992, Tan et al. 1998). As the meristem diameter increased to approximately 3000 μm (42 DAP) the magnification was reduced to 10x to allow the field of view to encompass the shoot apex. This reduced the accuracy to ± 50μm.

MORPHOLOGICAL DEVELOPMENT SCALE FOR THE SHOOT APEX

The morphological development of the shoot apex was recorded using a previously described scale (Tan et al., 1999a). Some modification of the scale was necessary to allow a more precise identification of the stages based on the appearance of the different branch orders, making the classification less subjective (Table 2). For example, Stage 5 of the scale is described as "Further development of numerous bract and floral primordia". While this is suitable as a descriptor of development, this definition in itself does not allow for an objective rating of this stage as bract and floral primordia production is occurring continually across different branch orders. Differentiating between stages based on the primordial branch orders provides a means of readily distinguishing 4, 5 and 6 of the original scale.

Table 2. *Stages of meristem development from the vegetative stage to buttoning, after Tan et. al.(1998).*

Stage
1 Vegetative The apical meristem is small and pointed, and surrounded by developing leaf primordia.
2 Transition The dome shaped apical meristem flattens while continuing to enlarge, and bract primordia are initiated. Primordia positioning begins transition to a spiral phyllotatic pattern.
3 Inflorescence initiation Floral initiation is evident by the development of II order floral branch primordia. No initiation of leaf primordia occurs from this point onwards.
4 Head Forming A second inner row of inflorescence branch primordia is initiated in an obvious Fibonacci phyllotaxis. III order branch primordia are few, or have not appeared yet.
5 Head Thickening Further rows of II order branch primordia appear accompanied by numerous III order branch primordia on their flanks. There is no elongation of the II order branch primordia, and no IV order branch primordia are present.
6 Head Thickening New II and III order primordia continue to appear and the production of IV order branch primordia also becomes apparent. Elongation of lower order primordia begins to occur.
7 Floral Initiation Elongation of the lower order branch primordia continues and initiation of the floral buds occurs as the highest order primordia begin differentiating the sepals.
8 Buttoning Inflorescence meristem is now 10-15mm in diameter.

STATISTICAL ANALYSIS

The coefficient of variation (CV) was used to describe the variation surrounding the means of each of the scale variables used in this study. The CV was calculated as

$$CV = \frac{s \times 100}{\bar{Y}}$$

where $\bar{Y}$ is the sample mean and s its standard deviation.

The CV was chosen as descriptor of variation as it is independent of the mean therefore allowing the comparison of variation between crops that may be at different developmental stages.

RESULTS

PLANT MORPHOLOGY DURING THE POSTULATED JUVENILE AND EARLY ADULT VEGETATIVE PHASE

At transplant the average seedling had a dry weight of 0.23 ± 0.02 g and was carrying 3.4 ± 0.07 leaves > 2 cm in length. Six out of ten plants had already lost one leaf and a further 3 out of ten plants was carrying an additional senescing leaf. By 14 DAP the average plant weighed 0.7 ± 0.11 g dry, was carrying 5.56 ± 0.38 leaves greater than 2 cm in length and had one vacant node due to senescence. By 28 DAP the average plant held 9.56 ± 0.24 leaves >2 cm and weighed 8.08 ± 1.3 g.

CHRONOLOGY OF INFLORESCENCE INITIATION

All plants remained vegetative and meristem diameter increased from transplanting to 14 DAP (Table 3). The mean (± SD) meristem diameter during the vegetative phase was 200 ± 56 μm (n = 397) and ranged from 96 μm to a maximum of 467 μm. By 28 days after planting (DAP), 2 of the 9 crops remained in the vegetative phase while the modal plants for the remaining crops were transitioning to, or had already transitioned (Crop's 4 & 8) to an inflorescence meristem. The mean meristem diameter at transition (Stage 2) was 432 ± 61 μm and 661 ± 125 μm at inflorescence initiation (Stage 3). The mean meristem diameter across crops at 28 DAP ranged from 292 - 864 μm (Table 3). The two vegetative crops (Crop's 6 & 9) had the smallest mean meristem diameters whilst those crops where floral initiation had already occurred had the largest meristem diameters (Table 3). The majority of plants across crops passed through the floral initiation stage between 28 and 42 DAP and at the end of this period the average meristem diameters ranged from 1029 – 7770 μm (Table 3). At this time the modal plants for each crop were at either stage 5 or 6, however Crop 6, still having the smallest mean meristem diameter was at stage 4. By 56 DAP the modal plant meristem was at Stage 8 with mean meristem diameters ranging from 8.17 – 28.51 mm, Crop's 6 and 4 having the smallest and largest diameters respectively. The average time between transition and buttoning was 28 days.

Table 3. *Mode and mean of the morphological stage and the meristem diameter (mean ± SD) at 14 day intervals across 9 crops from transplanting to buttoning.*

	0 DAP			14 DAP			28 DAP			42 DAP			56 DAP		
Crop	Modal Stage	Mean Stage	Meristem Diameter (µm)	Modal Stage	Mean Stage	Meristem Diameter (µm)	Modal Stage	Mean Stage	Meristem Diameter (µm))	Modal Stage	Mean Stage	Meristem Diameter (µm))	Modal Stage	Mean Stage	Meristem Diameter (µm))
1	1	1.0	164±24	1	1.0	215±19	2	2.1	478±81	5	5.0	2630±627	8	8.0	13354±3458
2	1	1.0	157±25	1	1.0	199±25	2	2.0	395±38	5	4.8	2115±604	8	8.0	10378±2029
3	1	1.0	156±17	1	1.0	218±40	2	2.2	429±101	5	5.3	3102±826	8	7.5	12924±6198
4	1	1.0	159±21	1	1.0	283±34	3	3.3	864±191	6	5.9	5770±1464	8	8.0	28508±1892
5	1	1.0	155±24	1	1.0	223±23	2	2.2	478±75	5	5.0	3283± 997	8	8.0	17103±6315
6	1	1.0	162±24	1	1.0	168±31	1	1.0	292±46	4	3.9	1029± 211	8	7.7	8168±5242
7	1	1.0	153±19	1	1.0	260±27	2	2.2	495±94	6	5.5	3755± 866	8	8.0	18944±4154
8	1	1.0	166±16	1	1.0	224±27	3	2.9	563±86	6	5.8	4630±1713	8	8.0	19066±7150
9	1	1.0	151±15	1	1.0	183±25	1	1.0	321±41	5	4.5	1332±387	8	8.0	9079±2573

DESCRIPTION OF VEGETATIVE GROWTH IN CONTEXT OF FLORAL DEVELOPMENT

Plant dry weight accumulation lagged during the first 14 days after transplant (Figure 1A). An exponential increase in growth was however evident by 28 DAP, at which time most plants were transitioning or had already initiated an inflorescence. During this period the variability in plant dry weight increased to a peak, before declining between 28 and 42 DAP to a relatively stable level (Figure 1B). A rapid change in relative growth rate occurred from 14 to 28DAP when most crops were transitioning, thereafter declining during the remainder of the crop cycle (Figure 1). The lag phase in plant dry weight accumulation and the subsequent rapid increase in RGR between 14 and 28 DAP was largely due to transplant shock. The large proportion of variability in RGR at 14 DAP as evidenced by the standard error of the mean was due to a correspondingly large difference in growth rates between crops, probably as they recovered from transplant shock at different rates. This variability rapidly decreased during 14 to 28 DAP suggesting that the majority of plants had by then adjusted to their new environment.

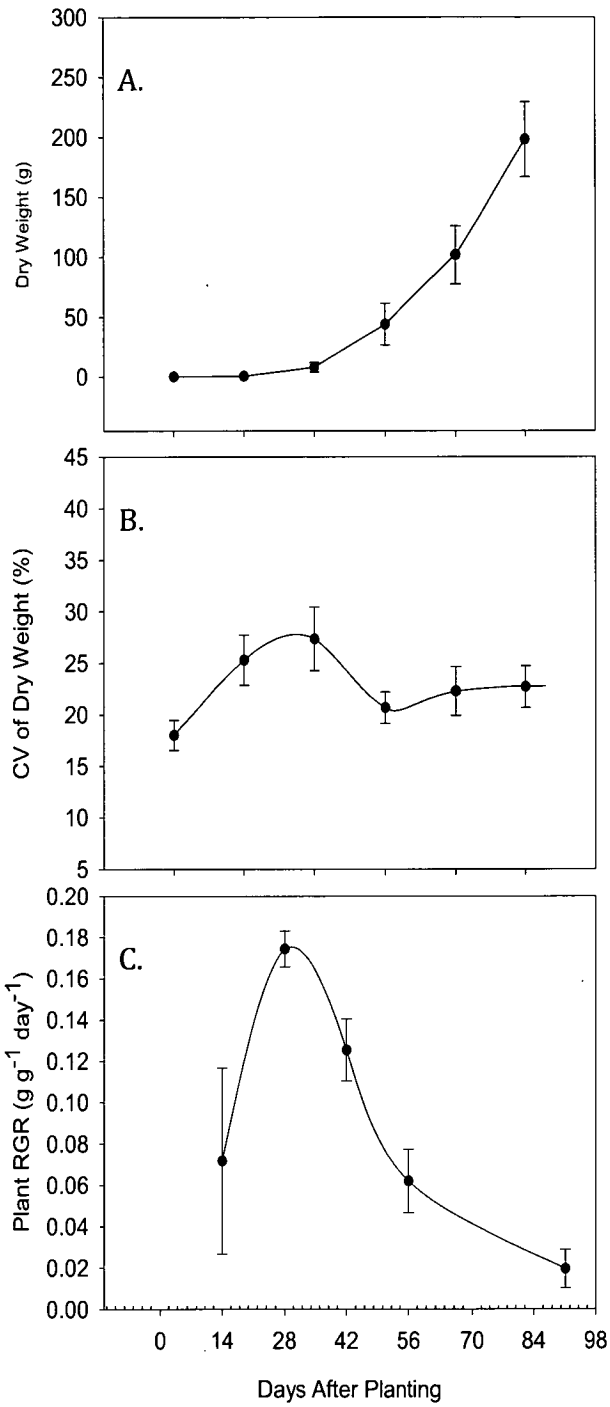


Figure 1. ***(A)*** *Mean dry weight accumulation,* ***(B)*** *the associated coefficient of variation and* ***(C)*** *relative growth rate from 0 to 96 days after planting. Error bars = SD.*

The variability in dry weight accumulation is presented in Figure 2, and it can be seen that the variability introduced at the establishment of each crop, continued for the rest of its cycle, and even though growth rates were similar, large variations in final yield and the time to harvest occurred. Crop's 6 and 9, the crops with the slowest floral development, also had the lowest growth rates during floral initiation and the longest times to harvest (Figure 2). Crops 4 & 8 the earliest to initiate flowering had comparatively high growth rates. This general relationship between plant growth rate was evident in a logistic regression analysis between plant dry weight and the floral status of plants at 28 DAP (Figure 3). Using plant dry weight and floral initiation defined as transition (Stage 2), the model

logit (p) = 1.122 plant dry weight - 4.78

correctly predicted a vegetative state in 73 % of cases, a floral state in 96% of cases, having an overall capability of 90 %. The probability of a plant having

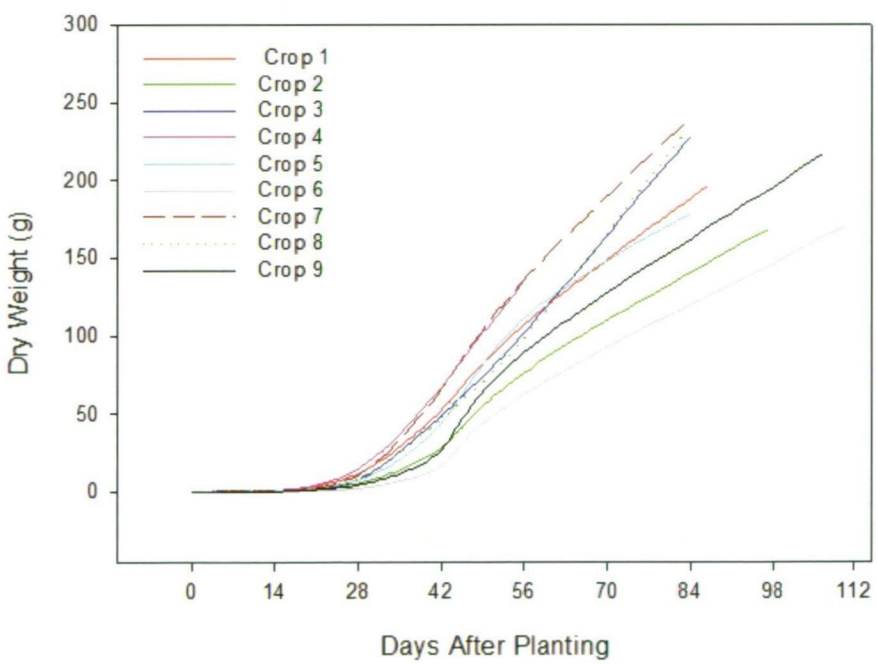


Figure 2. *Increase in plant dry weight for each crop from transplanting to harvest.*

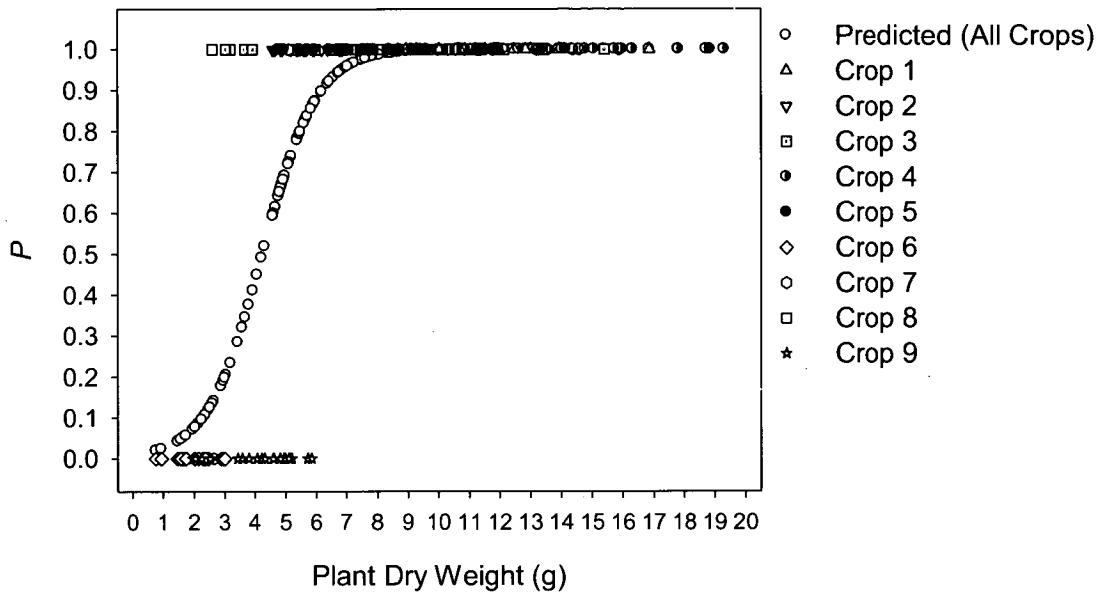


Figure 3. *Logistic regression demonstrating relationship between the probability of inflorescence initiation (defined as any plant that had transitioned) occurring at 28 DAP and plant dry weight.*

initiated an inflorescence increased three fold (exp $\beta = 3.07$) for each 1 g increase in plant dry weight, with 50% of the plants predicted to have started flowering at a dry weight of 4.26 g.

DESCRIPTION OF VARIATION IN PROGRESS TOWARDS FLOWERING

Meristem diameter increased from transplanting onwards with the rate of expansion increasing until 28DAP after which a steady rate was maintained until 56 DAP. The mean CV amongst crops began increasing from 14 - 28 DAP when the majority of crops were transitioning or had reached a reproductive state (Figure 4A). The variability in meristem diameter continued to increase until 42 - 56 DAP as the modal plant in each crop progressed from late curd development to buttoning. The spread in the mean CV between the crops (Figure 4B) also increased with time from transplanting onwards with that of crops 3 and 8 being particularly high at 42 and 56 DAP. The pattern of variation in the CV over time

was different for each crop, with some crops depicting high variability between dates (Figure 4B, Crop 2) and others a relatively steady increase (Figure 4B, Crop 3).

The CV in head diameter at harvest ranged from 10.2% (Crop 9) to 27.6% (Crop 1; Table 4). This level of variation of head diameter at harvest was lower than the variability in meristem diameter at the end of the floral initiation phase (Stage 8),

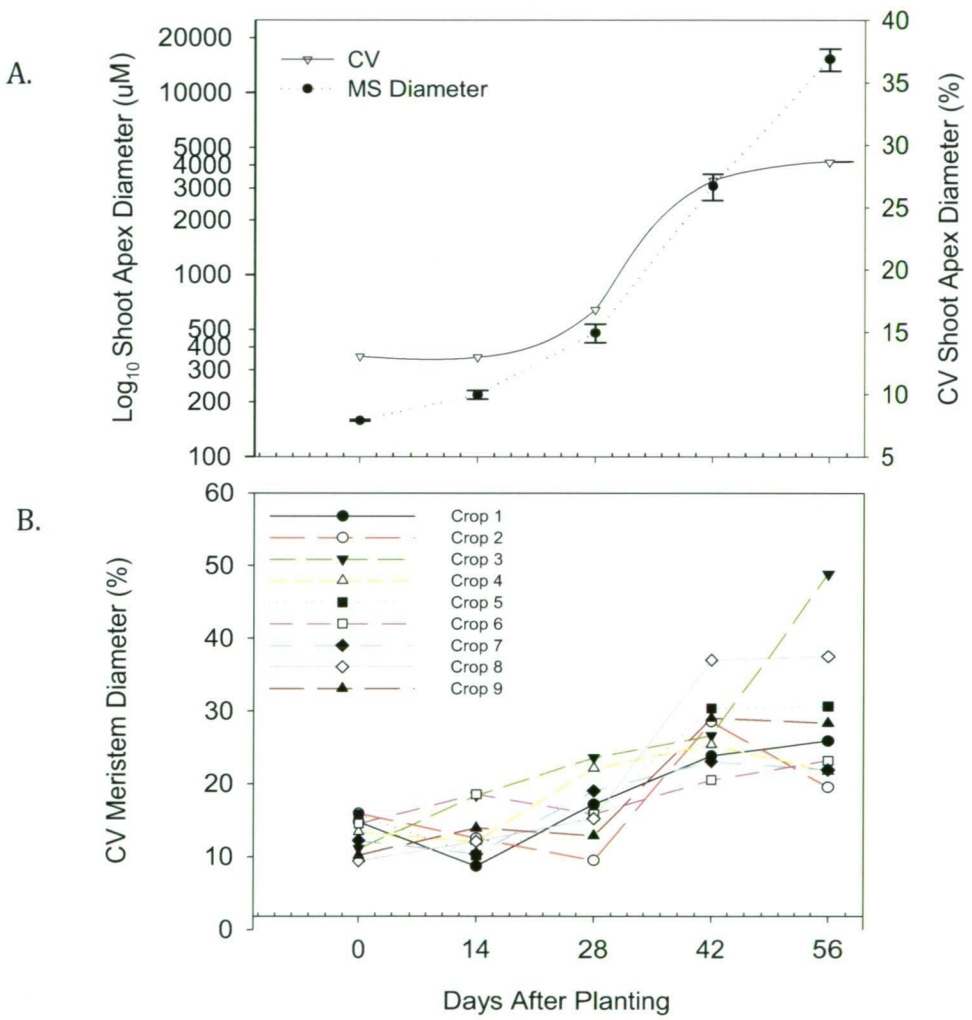


Figure 4. (A) *Log_{10} mean shoot apex diameter and morphological stage (left axis) and the associated coefficients of variation (right axis) from 0 to 56 days after planting. Error bars = SEM.* ***(B)*** *Coefficient of variation of the mean meristem diameter (n=20) for each crop over the same time span.*

which ranged from 20-50% (Figure 4B). The mean CV for all crops at harvest was however similar to that at 28 DAP. The variability in mean head diameter of each crop at harvest was linearly related to the variability in the mean meristem diameter ($r^2 = 0.49$, $P \leq 0.05$) and head dry weight ($r^2 = 0.57$, $P \leq 0.05$) at 28 DAP (Figure 5). In both cases this relationship was positive, with the variability in head size increasing with the variability in meristem diameter at 28 DAP. Neither head diameter nor dry weight at harvest was linked to the variability in meristem diameter at any other crop stage prior to inflorescence initiation or after this during curd development.

Table 4. Variation in head diameter at harvest.

Crop Number	Mean Diameter	Standard Deviation	CV
1	151	42	27.6
2	152	22	14.3
3	135	34	25.3
4	168	28	16.7
5	116	20	17.1
6	155	23	14.7
7	166	28	16.7
8	187	30	15.8
9	179	18	10.2
Mean CV			17.6

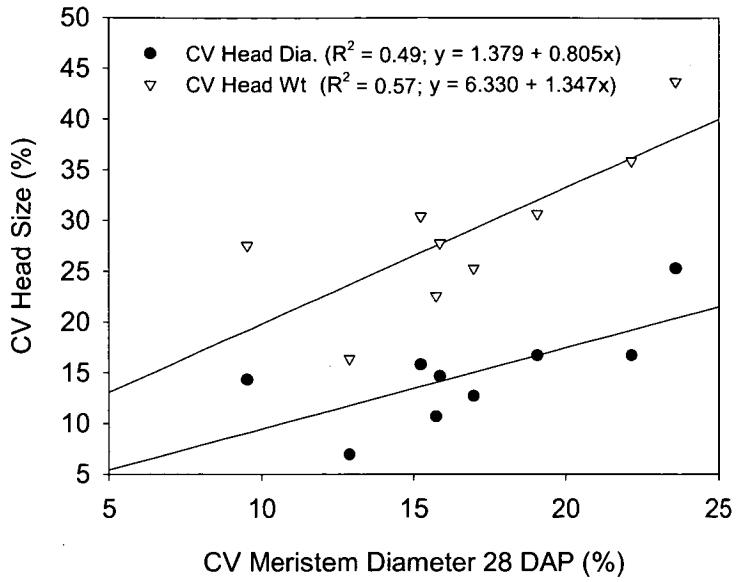


Figure 5. *Regression between the coefficient of variation for meristem diameter at 28 DAP and head size expressed as diameter and dry weight.*

DISCUSSION

This study highlights the importance of early crop life in broccoli production as variability in this stage of development is linked to variation in head diameter, a key measure of maturity at harvest. While variation in meristem diameter increased from 14 DAP to a peak at 42-56 DAP, it was the variability in meristem diameter at 28 DAP, that explained 49 - 57% of the variability in head size at harvest. As the majority of plants were transitioning at this stage, and the size of the meristems observed was similar to that previously linked to floral initiation (Wurr et al., 1995), it follows that the variability in floral initiation is also linked to the variability in harvest maturity. From this, one can conclude that the events leading up to transplanting, and the conditions during the first 28 days after this, have a significant bearing on the spread and timing of harvest events required to take broccoli heads at their optimal stage of maturity.

While it has previously been reported that variability in the time from planting to floral initiation is less than that of floral initiation to harvest (Wurr et al., 1991), when the coefficient of variation (CV) for these periods is calculated for 'Corvet' across years using the data presented, the variability was 21 & 20 % respectively. Thus the variation in the duration of these periods prior to and after initiation was roughly equal. Although time in days is a different measure of the plants progress towards flowering and harvest maturity, the levels of variation prior to initiation were thus similar to that of this study when measured as the coefficient of variation.

A number of models have indicated that head size at harvest is largely determined by temperature after floral initiation (Grevsen, 1998, Mourao and Brito, 2000, Wurr et al., 1991, Tan et al., 2000a), and it can therefore be argued that differences in the timing of this event can explain a large proportion of variability in head size at harvest. This provides further support for the assertion that the variation in floral initiation makes a significant contribution to variability in harvest maturity. Similarly for cauliflower, half the variation in harvest duration can be explained by the variation in the duration of curd initiation and the temperature experienced during curd development (Booij, 1990b).

The coefficient of variation for head diameter declined between initiation and harvest, as the variability observed in this latter stage was less than the peak level of variation observed for the shoot apex diameter during late curd development at 42-56 DAP. This is possibly an artifact of harvest maturity, as heads approaching the optimal time of harvest are also likely to be approaching their maximum diameter prior to the elongation of the II+ order branches and physiological maturity. Additionally, as diameter increases, each unit increase in diameter requires a higher level of assimilates to produce, and it likely that the rate of diameter increase slows with chronological age. These two factors combined would act to effectively reduce the earlier differences in maturity when this is evaluated as head diameter.

The influence of this early period of crop development on harvest maturity is likely to be related to both floral evocation and the rate of meristem development. If senescent leaves are included, approximately 60% of the seedlings at transplanting would have had a cumulative 4 or more leaves greater than 2 cm in length, this corresponding to the postulated end of juvenility (Wiebe, 1990). Also, as the seedlings were produced outside at ambient temperatures, they would have received sufficient chilling for vernalisation both before and after transplanting (Fontes et al., 1967). The exponential increase in meristem diameter and the absence of a lag phase from transplanting onwards (Wurr et al., 1995) provides firm evidence that evocation has occurred close to transplanting. However, as the variation in meristem diameter at transplanting or at 14 days after transplanting was not linked to the variability at harvest, this would suggest vernalisation has occurred during establishment. Further evidence that evocation has occurred around the time of transplanting, is the plant weight at which 50% of the seedlings had undergone transition. Based on the logistic regression with plant weight, the transition stage (Stage 2) would have been achieved between 14 and 28 DAP, indicating that this event had occurred prior to this period. Thus the time at which the seedlings were being planted out may have coincided with the important process of floral evocation. Consequently both seedling nurture leading up to transplant and conditions during establishment are of importance.

Plant to plant variability in the timing of floral initiation may have been introduced by genetic differences in the requirements for evocation. Temperature via vernalisation is the primary signal for evocation in broccoli (Friend, 1985), and in some varieties thermal time can be used to explain up to 89% of the variation in the time from seedling emergence to initiation. In other varieties such as 'Marathon' used in this study, variation in this cultivar, presumably genetic, demotes thermal time to a poor predictor of inflorescence initiation (Tan et al., 2000b). This variability may also extend to the requirements of vernalisation, and thus the inherent genetic attributes of the variety used in this study may have contributed significantly to the variability observed in meristem diameter during transition.

The increased variability during floral initiation and inflorescence development between individual plants and between sites may also have been generated by secondary or tertiary factors encountered by the seedlings during establishment. Differences in the micro climate experienced by individual seedlings created by variable soil to root contact, moisture availability, soil structure and nutrition may have delayed or suppressed the primary response to vernalisation in some plants (Bernier and Perilleux, 2005). Other factors such as transplant and pest damage may also have interrupted individual plant response to vernalisation, this leading to increased variability in floral initiation between plants.

The difference in the timing of floral initiation may also have been exacerbated by growth rate, as slow growing crops appeared to have delayed development, whilst those crops with higher growth rates appeared to advance farther with respect to inflorescence development. This observation is also illustrated by the relationship between plant weight and the likelihood of floral transitioning, with smaller plants having a lower likelihood having initiated flowering. This is possibly because crops or plants with slower growth rates will be smaller in relation to plants of the same cohort with higher relative growth rates, and are thus likely to be delayed in the attainment of the critical meristem diameter at which transitioning occurs. The 14 day period after transplanting was characterised by a low but highly variable relative growth rate between sites. But by 28 days after transplanting relative growth rate had peaked and variability was at its lowest. This pattern is probably a reflection of the differences induced by transplant shock and diversity in the recovery time associated with this. Thus the high level of variability in relative growth rate would magnify differences in plant to plant variability with respect to floral initiation that may have been induced by transplant shock.

There are a number of viable opportunities that might be pursued to minimise the variation encountered during transplanting and establishment. Holding broccoli seedlings in the tray cells until resource limitations constrain growth is one such option, as this can improve harvest uniformity. However, the increase in uniformity is also accompanied by a decrease in yield making this option less attractive (Damato and Trotta, 2000, Wien, 1997). Minimising transplant shock using best practices such as even soil preparation and irrigation during

establishment also provides some potential to minimise any variability in floral initiation that might be introduced through a heterogeneous environment with pockets of adverse soil moisture, soil structure, or nutritional status. As plant genetics is also likely to play a significant role, the use of cultivars with low levels of variability in the expression of the genes that determine floral evocation would also be advantageous.

In summary, variation in meristem diameter, used in this study as measure of a plants progress towards flowering, began to increase at the time of floral transition. While this variation continued to increase beyond 28 DAP it was variation at this time that explained a significant portion of the variability observed at harvest. The variation in floral initiation may have arisen from a variety of sources, both genetic and environmental. The findings of this study will allow future research to focus on these sources during early crop development, and may well provide the understanding necessary to reduce the number of harvests that are currently required to harvest terminal broccoli heads at their optimal stage of maturity.

CHAPTER 6

HOLLOW STEM IN BROCCOLI

The development of stem cavities is a common physiological disorder of both broccoli (*Brassica oleracea* L. var. *Italica*) and cauliflower (*Brassica oleracea* L. var. *botrytis*). Hollow stem severity may range from a fine split or cavity limited to a small section of a stem, to a large well developed cavity that extends almost the entire length of the stem. The presence of brown tissue that is sometimes associated with the walls of the cavity, and the potential for the post harvest entry of both dirt and disease, poses quality issues for both fresh and processing markets. As hollow stem can impact large proportions of a crop, this disorder has a significant impact on the net yield. While a number of studies looking at agronomic, genetic and environmental factors that influence the development of hollow stem have been undertaken, the underlying physiology contributing to the development of hollow stem is not understood.

EARLY REPORTS OF HOLLOW STEM

The earliest modern reports of stem cavities and the browning of stem tissue were reported circa 1918 in cauliflower crops grown in the Catskill Mountain region, New York State, USA, however formal investigations did not begin until the early 1930's (Hartman, 1937, Dearborn et al., 1936, Dearborn and Raleigh, 1935, Chupp and Horsfall, 1933, Dearborn, 1942). The disorder was referred to locally as "browning' or 'red rot' and was characterised by internal stem browning, the formation of stem hollows and discolouration of the head surface. The discovery that the application of boron as borax remedied this disorder in both cauliflower and rutabagas led to trials investigating the effects of boron deficiency on a number of other species including broccoli 'Italian Green Sprouting' (Chandler,

1940). Boron deficiency was first observed as epinasty and rolling of the leaf margins followed by the senescence of older leaves. Leaf loss was followed by the appearance of small swellings on the stem and ventral surface of the petiole. The ventral swellings on the petiole, which later became corky, were also accompanied by longitudinal cracking, again mostly on the underside of this organ. Vegetative plants were stunted, had brittle leaves and increased lateral branching while those plants with developing heads exhibited malformed heads with browning and eventually loss of the beads.

During this period there was already some disagreement (Chandler 1940) as to whether stem cavity formation in cauliflower was entirely associated with boron deficiency. Hartman (1937) noted that on Long Island hollow stem in cauliflower was quite prevalent yet even the most severe cases did not have any internal pith discolouration. Despite the prevalence and severity of hollow stem on the island, 'browning' had not been reported. He also provided anecdotal evidence of differences in the incidence of stem hollowing between cultivars, and a tendency for the disorder to increase under higher nitrogen rates. However, after conducting glasshouse and field studies, he concluded that stem browning was also the primary symptom of a boron deficiency, which may or may not be accompanied by surface browning and / or stem cavities. Chandler (1940) noted that while other investigators also believed hollow stem was caused by a boron deficiency, he found that in at least one cauliflower trial no apparent relationship existed between the two, and that hollow stem still occurred even at the extremely high rate of 56 kg B ha^{-1}. When hollow stem did occur in conjunction with a boron deficiency, his observation was that the cavity in both cauliflower and broccoli was always surrounded by brown or watery tissue. Others also reported stem hollowing in the absence of tissue discolouration (Dearborn and Raleigh, 1935).

From these earlier studies, and other subsequent reports (Benson et al., 1961, Petracek and Sams, 1987, Shelp and Shattuck, 1987) it is apparent that while boron deficiency was the earliest reported cause of hollow stem symptoms in Cole crops, the reported effects in controlled boron deficiency trials include other symptoms and morphological deformations beyond stem cavitation. The presence of hollow stem in field grown plants without these additional symptoms, and

particularly in plants growing in soil with high boron concentrations, is evidence against a causal role of boron deficiency in the development of the disorder. The debate on the role of boron deficiency as the primary cause of stem hollowing in the field had therefore clearly commenced in the earliest documented modern scientific studies of the disorder, and that debate has continued in the literature to the present day.

BORON

THE ROLE OF BORON IN THE PLANT

An understanding of the role of boron in plant metabolism is crucial to the boron hypothesis, as it may shed light on a mechanistic theory on how a deficiency might lead to the development of hollow stem. Boron has been recognised as a constituent of vegetative matter since 1857 and after numerous studies to determine its significance to plant life, was determined as an essential trace element in wheat in 1910 (Chandler, 1941, Agulhon cited by). This theory became more widely accepted after further convincing research by Warrington was published in 1923 Although its roles in specific instances of plant function have been identified, its primary role is to some extent still uncertain. Boron's prime importance in plant function appears to be its capacity to form monester and diester bridges between the *cis*-hydroxyl groups of various biochemical compounds (Shelp et al., 1995). It has been shown to play an important role in cell wall and plasma membrane function in addition to other plant metabolic processes such as organogenesis (Blevins and Lukaszewski, 1998).

MOBILITY IN PLANTS

In the early 20th century boron was thought to be immobile in plants, but some evidence, anecdotal at least, suggested otherwise. Chandler (1941) proposed that boron was not completely immobile in plants, noting that when moving rutabaga from sufficient to deficient supplies of B, new growth continued whilst the oldest

leaves senesced in much the same way plant leaves were known to do as they recycled the deficient element in response to a nutrient deficiency. Studies of boron content in broccoli tissue conducted 20 years later by Benson et al. (1961) supported this assertion. Despite these early hypotheses, until recently the general consensus has been that boron was only transported in the xylem stream and fixed in the apoplast once arriving at its destination, requiring that plants receive a continuous supply for adequate growth (Blevins and Lukaszewski, 1998). An example of the evidence used to support this conclusion is that in some species, up to 90% of accumulated B has been shown to be fixed in the cell wall (Blevins and Lukaszewski, 1998).

Some of the confusion arising over the mobility of boron undoubtedly arose due to the different capabilities amongst species to remobilise this element. It is only recently that boron's restricted mobility in the phloem and the mechanism by which this occurs in some plant species has been understood. Plants for which boron mobility has been demonstrated form *cis*-diol complexes with polyols and diols (eg, sorbitol, mannitol, etc) enabling its re-translocation via phloem throughout the plant (Brown and Shelp, 1997). Boron is relatively immobile in species that do not utilise these sugar alcohols in their sap stream.

The issue of boron mobility in broccoli is pertinent to the boron hypothesis as the ability to remobilise boron could remediate local deficiencies in developing plant tissues. Work by Barry Shelp at the University of Guelph has convincingly shown that under deficient conditions, or for that matter, even with sufficient supply, boron is retranslocated in broccoli from older source leaves via the phloem to those areas undergoing growth such as the developing inflorescence (Shelp, 1988, Shelp, 1990, Marentes et al., 1997). Approximately 73-93% of boron in the above ground plant parts is accumulated before the initiation of the inflorescence in broccoli however development of the reproductive tissue is still dependent on a continuous supply (Shelp et al., 1992). Remobilisation should to some extent mediate shortfalls in this supply during inflorescence development, the time at which hollow stem is initiated.

BORON DEFICIENCY SYMPTOMS IN BROCCOLI

The symptoms associated with imposed boron deficiencies, particularly severe deficiencies, are quite extensive (Table 1). Early descriptions of boron deficiency in broccoli by Chandler (1940) indicated deficiencies first became evident as brittle leaves that rolled and developed epinasty, followed by the loss of the oldest leaves. Following this, small ventral swellings which later dried and became corky, and longitudinal cracks appeared on the petioles. If the deficiency was applied prior to inflorescence initiation stem growth ceased and axillary branch production increased. If inflorescence development was underway when the deficiency was imposed, florets were aborted and the head became irregular in shape.

Benson et al. (1961) similarly described a wet or water soaked area on the stem usually subtending a leaf near the top of the plant as the initial symptom of boron deficiency in broccoli. This water soaked spots later dried and cracked with similar lesions appearing in the same region. Cracking later appeared in the whole upper section of the plant. As the deficiency progressed the interior of the stem became dry or corky, accompanied by dwarfed leaf tips, epinasty, leaf rolling and callus formation on leaf veins. Benson et. al. (1961) also noted a delay between the appearance of deficiency symptoms and the slowing of plant growth rate. Other studies have reported a similar complex of symptoms for broccoli and cauliflower (Table 1).

HISTOLOGY

Dearborn (1942) studied histological changes in cauliflower resulting from boron deficiency, observing that symptoms first occurred in the parenchyma cells of the pith and cortex regions. Isolated cells in the pith first enlarged and encroached on the intercellular spaces, within which a "mucilaginous or gummy" substance soon became apparent. This process was invariably associated with centre of the pith and restricted to the intercellular spaces, before affecting the outer regions. The substance, initially clear, gave the tissue a "watery soaked appearance" which soon turned brown in colour and at this point stained positive for lignin. As stem growth

occurred, a fine transverse split propagated through the gummed intercellular spaces. The above symptoms were also recorded in the younger vascular tissues, particularly phloem cells in the petiole, meristem and procambium.

HOLLOW STEM UNDER ADEQUATE BORON SUPPLY

The occurrence of stem cavities in broccoli has been reported in many studies where soil or tissue boron levels are considered adequate, and describe a relatively simple set of symptoms. The disorder is described as beginning in the centre of the stem soon after initiation of, and just below the terminal inflorescence, with elliptical transverse gaps that progressively enlarge to form interconnected cavities extending longitudinally throughout the stem. The cavities do not necessarily include brown tissue, although this may develop after harvest (Cutcliffe, 1972, Hipp, 1973, Wyatt et al., 1989, Zink and Akana, 1951, Shattuck and Shelp, 1987).

The majority of field studies that have considered hollow stem have simply defined the disorder as the presence or absence of cavities (Belec et al., 2001, San Bautista et al., 2005, Perniola et al., 1993, Gupta and Cutcliffe, 1973, Hipp, 1973, Shattuck et al., 1986, Coulombe et al., 1999, Zink and Akana, 1951, Gorski and Armstrong, 1985, Griffith and Carling, 1991, Peck and MacDonald, 1986, Brainard and Bellinder, 2004). In addition to recording incidence others have additionally rated the severity by length of the cavity (Damato et al., 1994, Everaarts and Putter, 2003, Damato and Trotta, 2000) or by means of an index (Shattuck and Shelp, 1987, Vigier and Cutcliffe, 1984). Tremblay (1989) estimated the severity of hollow stem by filling the voids of the head and stem tissue with water, and then measuring the volume of water retained with a measuring cylinder. In all of these instances it is notable that additional symptoms beyond cavity formation are either not mentioned or observed, suggesting that in many cases the disorder was viewed as consisting chiefly of the symptoms described above.

There are a number of studies that clearly illustrate an inconsistent relationship between boron deficiency and the appearance of stem cavities. Zink (1951) noted

the absence of bud deterioration associated with boron deficiency despite the occurrence of hollow stem in sprouting broccoli cultivars. Gupta and Cutcliffe (1973) recorded a high incidence of hollow stem in broccoli and cauliflower on four soil types ranging from 0.34-0.49 mg B kg^{-1}, but did not observe other symptoms consistent with a boron deficiency. Additionally the incidence of hollow stem did not respond to B applied at up to 4.48 kg ha^{-1}. Shattuck and Shelp (1987) also encountered significant levels of hollow stem in plants that were shown to have adequate tissue boron levels of 40- 47 mg kg^{-1}. Tremblay (1989) while running field trials across two sites found reduced levels of hollow stem in the trial with the lowest soil B content (0.09 mg kg^{-1}). Other studies have also concluded that supplementation with boron, nor soil or plant tissue boron concentrations are necessarily linked to the incidence of hollow stem (Everaarts and Putter, 2000, Zink, 1968, Scaife and Wurr, 1990, Gupta and Cutcliffe, 1975, Hipp, 1973, Griffith and Carling, 1991, Vigier and Cutcliffe, 1984).

In the absence of extensive boron deficiency symptoms, only one field study has demonstrated a reduction in stem cavity incidence in response to the application of boron (Batal et al., 1997). In this study on two boron deficient soil types in Georgia, USA (Tifton at 0.07 mg B kg^{-1}; Plains at 0.27 mg B kg^{-1}), soil-applied boron decreased the incidence of hollow stem in cauliflower 'White Empress' at both sites as rates were increased from 2.2 to 8.8 kg B ha^{-1}. Other than cavity formation, no other symptoms consistent with a boron deficiency were reported.

Table 1 *Symptoms and tissue B concentrations associated with boron deficiencies in broccoli and cauliflower as reported by various studies. The units of measurement reported in the original studies have been converted to mg kg^{-1} to ease interpretation.*

Asymptomous Tissue B concentration	**Symptom Level** Tissue B concentration	**Symptoms**	**Source**
		Broccoli	
		Glasshouse Study: Dwarfing of the entire plant. Brittle leaves, that are shorter and narrower and develop rolling and epinasty, followed by loss of oldest leaves. Small ventral swellings on petiole that later become corky and longitudinal cracking. Inflorescence bead loss and shape deformation. Reduced plant stature and death of apical meristem. Loss of pods in seeding plants.	(Chandler, 1940)
13-70 mg kg^{-1} (Leaf)		*Field Study:* No symptoms observed. 50% hollow stem.	(Gupta and Cutcliffe, 1973)
22.3-22.5 mg kg^{-1} (Leaf)	8.6-9.1 mg kg^{-1} (Leaf)	*Field study.* Leaf margin browning. Reduced growth and yield. *Glasshouse Study:* Uneven leaf yellowing, stunted growth. Some stem cavities.	(Gupta and Cutcliffe, 1975)
13.2-18.4 mg kg^{-1} (Shoot)	2.4 mg kg^{-1} (Shoot)	*Glasshouse study.* Leaf yellowing, stunted growth. Decreased yield. HWS soil B 28 mg kg^{-1}.	
		Glasshouse Study: Water soaked area of the outside stem which later dries and cracks. Pith corkiness. Dwarfed leaf tips, epinasty, leaf rolling and callus formation on leaf veins. Further stem cracking.	(Benson et al., 1961)
35 mg kg^{-1} (Stem) 45 mg kg^{-1} (Florets)	19 mg kg^{-1} (Stem) 23 mg kg^{-1} (Florets)	*Glasshouse Study:* Stem and lamina cracking (external). Brown stem tissue and small cavities of necrotic tissue.	(Shattuck and Shelp, 1987)

Asymptomous Tissue B concentration	**Symptom Level** Tissue B concentration	**Symptoms** – *Broccoli cont.*	**Source**
	5 mg kg^{-1} (leaf 12) 6 mg kg^{-1} (leaf 16)	*Glasshouse Study:* Depressed growth, chlorophyll levels and net photosynthetic rate. Scales and blisters on the stem. Scales on the base of the petiole in the higher leaves, progressively moving through to lower leaves. Thickening and chlorosis of the younger leaf tips progressing to all leaves. Petioles of the young leaves were split and blackened. Stem cavities with brown tissue.	(Petracek and Sams, 1987)
540-700 mg kg^{-1} (Oldest leaves) 260-570 mg kg^{-1} (4-6 Young leaves) 35-60 mg kg^{-1} (Stems) 45-68 mg kg^{-1} (Head florets)	39 mg kg^{-1} (Oldest leaves) 29 mg kg^{-1} (4-6 Young leaves) 19 mg kg^{-1} (Stems) 21-26 mg kg^{-1} (Head florets)	*Glasshouse Study:* Mild stem discolouration, corkiness and small necrotic spots. Leaf mid rib cracking. Reduced fresh weight of foliage.	(Shelp, 1988)
45-68 mg kg^{-1} (florets)	23 mg kg^{-1} (florets) 29 mg kg^{-1} (young leaves) 39 mg kg^{-1} (Old leaves)	*Glasshouse Study:* External stem and leaf mid rib cracking, necrosis of inflorescence pith. Discoloured pith tissue at 42 mg B kg^{-1} in floret tissue. Head colour change from blue green to dull green at 430 mg B kg^{-1} and marginal leaf yellowing, with the exception of YFEL at 720 mg B kg^{-1}.	(Shelp, 1990)
62-90 mg kg^{-1} (Shoot) 117-236 mg kg^{-1} (Old leaves) 66-77 mg kg^{-1} (Young leaves) 40-45 mg kg^{-1} (Florets)	8-9 mg kg^{-1} (Shoot) 25-48 mg kg^{-1} (Old leaves) 24-29 mg kg^{-1} (Young leaves) 7-13 mg kg^{-1} (Florets)	*Glasshouse Study:* Reduced plant fresh weight. Decreased diameter, deformation and browning of heads (floret death). Stem corkiness. Leaf and mid rib cracking. Brown pith discolouration, necrosis and hollow formations.	(Shelp et al., 1992)

Asymptomous Tissue B concentration	**Symptom Level** Tissue B concentration	**Symptoms**	**Source**
		Cauliflower	
		Field observations: Small discoloured spots on the curd, gradually spreading over the entire inflorescence. Head discolouration frequently preceded by pith darkening and degradation. Bitter taste. Inter-venial yellowing.	(Chupp and Horsfall, 1933)
		Glasshouse Study: Dwarfed plants with brittle leaves that roll, being more severe in younger leaves which also develop epinasty. Intermediate leaves may be rugose. Under severe deficiency young leaves have no blade and an enlarged midrib. Heads abort under severe deficiency. Under moderated deficiency head surfaces are watery or brown, and the pith similarly transparent or brown.	(Chandler, 1940)
		Field and Glasshouse studies: Small scattered spherical water soaked areas in the stem and branches that enlarge and unite. Followed by transverse, and then longitudinal cracking of the pith, and hollowing of both stem and inflorescence branches. Dull green, thickened, brittle, epinastic leaves, older leaves with yellow green apical margin. Ventral, proximal petiole blistering and dorsal surface of the midrib, which later burst creating small transverse and sometimes longitudinal cracks.	(Dearborn, 1942)
8 - 97 mg kg^{-1} (Leaf)		*Field Study:* No symptoms observed. 70% hollow stem.	(Gupta and Cutcliffe, 1973)
16.0-17.4 mg kg^{-1} (Leaf) 10.5-13.6 mg kg^{-1} (Shoot)	7.8-8.9 mg kg^{-1} (Leaf) 4.2 mg kg^{-1} (Shoot)	*Glasshouse study:* Reduced growth and yield, uneven yellowing of leaves and purple discolouration, upward curling of leaf margin. No stem cavities. *Glasshouse study.* Leaf yellowing, purple coloured cupped leaves. Stunted growth. (Glasshouse). HWS soil B 28 mg kg^{-1}.	(Gupta and Cutcliffe, 1975)
543 mg kg^{-1} (2 oldest leaves) 145 mg kg^{-1} (4-6 youngest leaves) 41 mg kg^{-1} (Head)	35-222 mg kg^{-1} (2 oldest leaves) 55 mg kg^{-1} (intermediate leaves) 48-53 mg kg^{-1} (4-6 youngest leaves) 21-26 mg kg^{-1} (Head)	*Glasshouse Study:* Leaf rolling (young leaves). Corking and cross hatching of upper leaf surface, midrib and petiole. Abnormal (loosely packed with some browning) or failed curd production. Pith browning, necrosis and the appearance of small lesions. Small longitudinal split in acute cases. Reduced yield.	(Shelp and Shattuck, 1987)

GROWTH RATE

Growth rate or associated factors such as increased plant spacing and nitrogen application, have been suggested as a possible cause of hollow stem in cauliflower and broccoli (Scaife and Wurr, 1990, Everaarts and Putter, 2000, Everaarts and Putter, 2003, Mullins and Straw, 1990, Wyatt et al., 1989, Tremblay, 1989, Zink and Akana, 1951, Gorski and Armstrong, 1985, Nieuwhof, 1969, Hipp, 1973). While many authors have suggested this link based on plant size, and correlations with increased planting density or rates of nitrogen application, there is only one study where more direct evidence using calculated growth rates has been supplied. In this study Everaarts and Putter (2003) manipulated growth rate in cauliflower using a range of planting densities and were able to show that in their trials at least, the incidence of hollow stem increased concomitantly with absolute growth rate (g day^{-1} fresh weight) of the stem.

Growth rate may not however explain the incidence of hollow stem in all cases, as noted by Tremblay (1989). In this study, hollow stem severity was significantly greater in the year where growth rates were lowest and maturity had been delayed by 15 days, this presumed to be a seasonal effect. In a subsequent study investigating the effect of N rates and the timing of application, Coulombe *et. al.* (1999) published data on hollow stem in broccoli 'Arcadia' grown at Ste-Croix L'Acadie in Canada. Hollow stem increased with N application rates made at 5 weeks at Ste-Croix in both 1996-97, and in 1996 at L'Acadie. Yet, in the second year at L'Acadie, despite a yield response to N application at 5 weeks, and higher total yields than the previous year, hollow stem incidence was minimal, and did not respond to increased N application. This would seem to indicate that a factor or factors other than growth rate as measured by plant yield are implicated in the development of hollow stem.

NITROGEN SUPPLY EXACERBATES HOLLOW STEM

A number of studies have convincingly imputed a link between the development of hollow stem and rates of nitrogen application in both broccoli and cauliflower. The incidence of hollow stem tends to increase with increasing rates of nitrogen (Vigier and Cutcliffe, 1984, Zink, 1968, Cutcliffe, 1972, Cutcliffe, 1975, Belec et al., 2001, Scaife and Wurr, 1990, Hipp, 1973, Tremblay, 1989, Coulombe et al., 1999, Babik and Elkner, 2002, Gorski and Armstrong, 1985). Some of these authors have suggested that this relationship is a result of increased plant size or growth rate resulting from increased nitrogen supply. Scaife and Wurr (1990), when comparing the incidence and severity of hollow stem in cauliflower under a factorial irrigation x nitrogen regime, found that the development of hollow stem was significantly correlated with plant fresh weight and leaf N content. They suggest that the development of hollow stem was more likely related to plant size or growth rate over nitrogen, and that its development was associated with growth during the latter stages of curd formation. Similarly, Kowalenko and Hall (1987) also concluded that nitrogen application during head development was important. They observed a high demand for nitrogen during inflorescence development of broccoli 'Premium Crop', and suggested that a split application timed just prior to 'bud initiation' may reduce the incidence of hollow stem, as opposed to total N applications made at transplanting.

A decrease in the incidence of stem cavity formation has been associated with delays in harvest maturity in a study conducted by Hipp (1973). This delay increased as nitrogen availability decreased, suggesting that the lowered incidence of hollow stem was again related to growth rate.

Although increased nitrogen supply tends to increase the incidence of hollow stem, some studies have reported no effect from increased N fertilisation (Shattuck and Shelp, 1987, Thompson et al., 2002), suggesting that other factors can override this response. The source of nitrogen used (Tremblay, 1989) and the timing of N applications (Coulombe et al., 1999) also affects the incidence of stem hollowing.

INFLUENCE OF BORON ON NITROGEN METABOLISM

Some authors have suggested that the boron may influence nitrogen metabolism in broccoli and that this may be relevant to the development of stem cavities in broccoli. Vigier and Cutcliffe (1984) when investigating the effect of nitrogen and boron on the incidence of hollow stem observed that boron availability may influence nitrate levels in the leaf tissue. In their study, at one location, the lower rates of B application (2-4 kg ha^{-1}) increased leaf nitrate levels, and the leaf nitrate to boron ratio was correlated with hollow stem incidence. Hollow stem peaked at 67% at 490: 1 and was lowest at 215: 1. Thus the NO_3^-: B ratio may somehow contribute to the development of stem cavities, although as with all correlations, this relationship in not necessarily causal.

Shattuck and Shelp (1987) observed an increase in elemental N of the youngest leaves in cauliflower when B levels in a nutrient solution system were increased. Later Shelp (1990, Shelp, 1988) provided evidence that both boron deficiency and toxicity increased the concentration of inorganic N (NO_3^- and NH_4^+) in broccoli tissues and decreased the concentration of glutamine and other amino acids in actively growing tissues. This and a decreased C:N ratio observed in vascular fluids led the author to concluded that B deficiency and toxicity may induce limitations to the carbon supply, influencing the conversion of nitrate to ammonium, and consequent assimilation of ammonium into amino acids.

HOLLOW STEM, PHOSPHORUS AND POTASSIUM

The application of essential mineral elements other than nitrogen can also influence the development of hollow stem in broccoli and cauliflower. Peck and MacDonald (1986) applied varying rate combinations of concentrated super phosphate and potassium chloride to broccoli 'El Centro' and cauliflower 'Imperial'. For both varieties, the interaction between these two fertiliser types influenced the incidence of hollow stem. The highest yields of broccoli across the three harvest dates were produced under 336 kg ha^{-1} super phosphate and 268 kg ha^{-1} potassium chloride. These rates also produced the highest incidence of stem

cavities. The cavities in this study were accompanied by dull brown tissue. San Bautista *et. al.* (2005) also noted that 'Marathon' yields were increased by a low K:N (1.19) and that under this treatment hollow stem incidence was also greater. It should be noted that the incidence of hollow stem observed in this study was in the order of 0-2%.

TRANSPLANTING

A delay in transplanting past an optimal stage can influence the development of hollow stem. In Policoro, Italy, the delayed transplanting 7 days past the 3 true leaf stage reduced plant weight, inflorescence diameter, yield of the terminal heads and the incidence and severity (cavity length) of hollow stem in 'XPH 4141' but not 'Gran Vert', and 'ML 423' (Damato and Trotta, 2000). Similar results were obtained in a second study investigating transplant cell size and delayed plantings, with negligible hollow stem observed in 'Gran Vert' or ML 423'. However, for 'XPH 4141', in addition to a reduction in stem hollowing as transplanting was delayed by 7 days, presentation of this disorder was reduced as transplant cell size decreased from 40 to 5 cm^3 (Damato et al., 1994). Holding period did not have a significant effect on marketable yield however individual head weights, and total yield (terminal and lateral heads) did decline, indicating that this response could possibly again be attributed to growth rate.

PLANTING DENSITY

Planting density has been linked to the development of hollow stem in broccoli in a number of studies (Zink, 1968, Cutcliffe, 1972, Cutliffe, 1975, Zink and Akana, 1951, Gorski and Armstrong, 1985, Griffith and Carling, 1991). In all of these studies the incidence of hollow stem increased with a reduction in planting densities. This trend appears to be consistent across both cultivars and seasonal effects (Cutliffe, 1975). Hollow stem has been recorded at densities ranging from 28 000 plants ha^{-1} (Griffith and Carling, 1991) to 136 000 plants ha^{-1} (Hipp, 1973). Although planting density appears to have a strong influence on the development of stem cavities, the

effect of increased plant spacing on hollow stem can be overridden by both genetic and environmental factors as observed by Griffith and Carling (1991). In cauliflower, decreasing planting density from 52 000 plants ha^{-1} to 26 000 plants ha^{-1} or less led to a significant increase in the incidence of hollow stem and the length of the ensuing cavity (Everaarts and Putter, 2003).

STEM SIZE

When comparing the mean stem diameters of plants affected and unaffected by hollow stem, Zink (1968) reported that hollow stem incidence was associated with plants that had larger stem diameters at a 152mm cut. These results supported earlier observations in three varieties of sprouting broccoli ("Early', 'Midway' and 'Medium'), that the average stem diameter for hollow stem affected plants was greater than that of the population samples (Zink and Akana, 1951). Stem diameter increased as densities ranged from 193 750 plants ha^{-1} to 36 904 plants ha^{-1}. Stem cavity formation has also been attributed to the rate of growth in the stem of cauliflower. In cauliflower 'Fremont', Everaarts and Putter (2003) established a linear relationship between the absolute growth rate of the stem, measured as fresh weight, and the incidence of hollow stem.

IRRIGATION

Irrigation was shown to accentuate the influence of nitrogen on the incidence of hollow stem in a Polish study of 'Marathon' (Babik and Elkner, 2002). In this study, hollow stem did not respond to increased nitrogen rates under dry land conditions, with one minor exception. Yet under irrigation, hollow stem incidence and plant weight increased with increasing rates of nitrogen, and the time to harvest was decreased by an average of 6 days, indicating a possible increase in absolute growth rates. San Bautista (2005) also noted a slight increase in stem cavity formation incidence in 'Marathon' under full replacement of evapotranspiration when compared to 45% replacement, under which yield was depressed.

GENOTYPE AND ENVIRONMENTAL RESPONSE

A number of studies have illustrated a link between genotype and susceptibility to hollow stem (Cutcliffe, 1972, Shattuck et al., 1986, Cutliffe, 1975, Shattuck and Shelp, 1987, Shelp et al., 1992, Perniola et al., 1993, Damato and Trotta, 2000, Griffith and Carling, 1991). Whilst some broccoli cultivars are consistently susceptible or tolerant to hollow stem in changing seasons, other varieties are quite variable in their response, indicating that genotype has varying levels of control in the development of this condition. 'Commander' was shown to be consistently resistant (Shelp et al., 1992) to the development of this disorder whilst others such as 'Green Comet' exhibit consistently high levels of hollow stem despite seasonal differences, indicating that the response for these cultivars is largely genetic (Shattuck et al., 1986). Other cultivars such as 'Green Valiant' (Shattuck et al., 1986) and 'Harvester' (Cutliffe, 1975) display significant instability, with levels of hollow stem being heavily influenced by the growing environment. Shelp et. al. (1992) suggested that the tolerance of 'Commander' to hollow stem was due to its increased tolerance to boron deficiency. The authors also conclude that the mobility of boron was different between cultivars (due to genetic variation) and that although uncertain, the tolerance of 'Commander' relative to the other cultivars tested, was possibly due to an increased ability to remobilise boron from older plant parts.

Seasonal changes, within and between years, can have a significant bearing on the development of hollow stem. This effect has been evident in a number studies where identical or similar trial designs have been conducted at the same locations and cultivars over different years (Scaife and Wurr, 1990, Belec et al., 2001, Shattuck et al., 1986, Cutliffe, 1975, Hipp, 1973, Coulombe et al., 1999), despite the plants being subject to treatments such as high nitrogen applications, that in other years produced higher levels of hollow stem. Zink (1968) provided anecdotal observations that Californian broccoli crops maturing over the warmer months have a greater incidence of hollow stem than those maturing in the cooler months of the year.

In the second year of a study across two sites, Belec et al. (2001) report that the incidence of hollow stem was reduced at each, most notably 3-4 times at one site in comparison to the previous year. Not only was the response reduced at this site, the response to N application observed in the first year was comparatively low in the second. It should be noted that any effect across the two years could also be attributed to other site factors (e.g. irrigation management), although the authors make no reference to any such circumstance.

In a 3 year study at Simcoe, Ontario, Canada, Shattuck et. al. (1986) investigated the genotype x environment interaction to measure the stability of yield and hollow stem incidence across six cultivars.. This study found a main seasonal effect and apart from one cultivar in one year, there was no difference in susceptibility between cultivars within a year. A pooled analysis over the three year period indicated that all of the cultivars except 'Green Comet' exhibited average to below average stability, indicating the growing environment had a noteworthy impact on hollow stem incidence, and that each cultivar differed in the magnitude of its response to environmental changes. These findings are to some extent confounded by a change in planting density between the first and last two years of the experiments.

Thus it would seem the underlying mechanism responsible for the development of the hollow stem disorder is influenced by both genotype and the environment.

CONCLUSION

It is evident that in many studies of hollow stem, particularly those conducted under a sufficient supply of boron, only the formation of stem cavities and the browning and splitting of the pith tissue are consistent with that observed in boron deficiency studies. Extensive hollow stem in field crops is commonly observed in the absence of wet stem lesions, external stem cracking, corkiness or cracking of the leaf petiole, leaf browning or any notable discolouration or deformities of the head, symptoms reported for boron deficiency in broccoli. While there is no doubt that boron deficiency in broccoli and cauliflower leads to pith degradation and

necrotic cavities, it may be that a boron deficiency may share these symptoms with a separate disorder. Because stem cavities may be a common symptom, confusion may have arisen as to the identity of the true underlying cause of hollow stem in broccoli grown on boron sufficient soils.

It has been noted that the inability to directly link soil and tissue B levels to the incidence and severity of hollow stem in broccoli and cauliflower, may be due to the influence of external environmental influences. Under this scenario, boron deficiency is still the primary mechanism attributed to stem cavity formation, however the response is mediated by external environmental influences (Shattuck and Shelp, 1987, Shelp and Shattuck, 1987). It is also possible that a boron deficiency may only exist at a localized tissue level in plants that otherwise are in full receipt of a sufficient boron supply (Shelp et al., 1992), a role that is much harder to detect. In this instance it may be that some physiological mechanism, induced by rapid growth under favourable conditions, may influence the mobility and distribution of boron in broccoli. One possible mechanism is the reallocation of plant assimilates from plant parts other than the oldest leaves. Under deficient conditions, remobilisation of B to areas of the plant where rapid growth is occurring, such as young leaves and the inflorescence, could drive, or be a part of, autolysis of the pith tissue, a mechanism suggested for the aetiology of stem hollowing in other plant species (Carr and Jaffe, 1995). A shortfall in photosynthates and mineral elements may result in the autolysis of stem pith tissue, the cells of which are likely to store starch. This remobilisation of carbohydrate reserves would remedy the shortfall in assimilate and element supply, particularly under conditions that promote disproportionate growth rates. Additionally, a localised shortfall in mineral elements such as boron, might elicit a similar response.

By far the weight of literature would seem to suggest that the development of hollow stem finds it origin in a mechanism affiliated with growth rate, particularly that observed in field studies in the absence of brown tissue. These studies consistently illustrate that factors resulting in increased growth rates such as improved nutrition and irrigation, decreasing plant density, and planting techniques that maximise establishment generally increase the incidence and

severity of stem cavities. Additionally different measures of growth rate such as stem size, yield and plant size have been correlated with the incidence of hollow stem. The fact that increased growth rate does not always increase hollow stem would suggest that the response may only apply to a particular facet of growth or that it is mediated by other factors. The discriminate response to growth rate might be explained by a restriction to a particular stage of development, for instance inflorescence development, as this appears to be the stage at which hollow stem is initiated. Alternatively, the patterning of growth across the stem tissue may be of importance. Griffith and Carling (1991) postulated that formation of stem hollows in broccoli could be initiated by differential radial strain across the stem leading to the development of internal stem cracking, and that this mechanical stress could be related not so much to growth rate, as to the size of the inflorescence.

The disparity in results in all studies to date has demonstrated that neither a boron deficiency or growth rate per se. provide an adequate explanation for the development of hollow stem in the absence of a significant boron deficiency. It is likely that an understanding of the development of stem cavities in both broccoli and cauliflower requires the development of specific mechanistic hypotheses, particularly those suggested for growth rate.

CHAPTER 7

THE AETIOLOGY OF HOLLOW STEM

INTRODUCTION

The most detailed descriptions of stem cavity development in broccoli and cauliflower have been derived from studies on boron deficiency (Dearborn et al., 1936, Hartman, 1937, Benson et al., 1961). While it is evident that a boron deficiency does lead to the development of necrotic cavities in broccoli under controlled conditions (Shelp, 1990), it is still possible that the cavity development observed in other field trials is different to that observed in these studies. As such, a detailed description of the aetiology involved in cavity formation will help determine if the symptoms previously described are consistent with that occurring under field conditions. As there is also evidence that other mechanisms may be responsible for the occurrence of stem cavities, an improved understanding of the symptoms associated with this may provide further evidence for the involvement of other processes.

Previous studies of hollow stem in field grown broccoli in which the boron supply has not been questioned, have described the disorder as beginning at the same time as the appearance of, and just below the terminal inflorescence (Zink, 1968, Cutcliffe, 1972). Little is said about the initial fissure other than it appears to begin as a small elliptical crack, split or vertical cracking in the inner pith tissues (Shattuck et al., 1986, Griffith and Carling, 1991). These elliptical cavities then continue to enlarge, eventually coalescing to form a hollow stem (Cutcliffe et al., 1968, Shattuck et al., 1986, Zink and Akana, 1951). Severe cavities may extend through the stem to the surface of the head in cauliflower (Scaife and Wurr, 1990). The pith tissue may also exhibit a brown discolouration (Shattuck et al., 1986) however this does not always accompany cavity development (Cutcliffe, 1972, Zink

and Akana, 1951, Shattuck and Shelp, 1987), and may not develop until after harvest (Zink and Akana, 1951). In some cases it is also specifically stated that other symptoms associated with boron were not observed (Zink and Akana, 1951, Griffith and Carling, 1991).

There is a clear discrepancy between the description outlined above and that described for stem cavities under controlled boron deficiency studies. While both cavity development and the occurrence of necrotic tissues are encapsulated in the aetiology of boron deficiency, the set of symptoms associated with this disorder is more extensive (Petracek and Sams, 1987, Chandler, 1940, Benson et al., 1961, Shelp, 1990). This suggests that stem cavity development may be a common symptom shared between two separate disorders.

Gaining a greater understanding of the symptomatology of hollow stem will provide further insight into the possible origin of this disorder. For instance, it has been suggested that the relationship between planting density and hollow stem might be related to the final size of the inflorescence, as a consequence of radial strain developed in the stem (Griffith and Carling, 1991). If this is the case, then evidence associated with tissue failure such as cell separation of fracture should be evident using microscopy, or additionally, other gross symptoms associated with tissue failure might be visible at a macroscopic level.

The occurrence of hollow stem during early inflorescence development and its initiation close to this event at the top of the stem also raises the possibility that the reallocation of carbohydrates stored within the pith tissues may be responsible. In other horticultural species (beans and tomato) it has been proposed that the reallocation of carbohydrates is responsible for the development of pith deterioration (Carr and Jaffe, 1995). Under this scenario, the demand for assimilates created by the rapid growth of the inflorescence in some plants may exceed the capacity of the photosynthetic system. This short fall in capacity might then be met by the removal of starch stored in the central pith, and the eventual autolysis of these cells via programmed cell death undertaken to further bolster assimilate supply. If this hypothesis is correct, then starch levels in the stem pith

tissues should decline prior to the development of hollow stem under conditions where this is likely to develop.

Thus a comprehensive description of stem cavity development will provide a basis for comparison against those symptoms described for boron deficiency, and possibly provide evidence that additional or, alternative mechanisms such as mechanical tissue stress might be responsible. The objective of this study was to describe the development of hollow stem in field grown broccoli and compare the observed symptoms with that previously described for stem cavity development in other studies. In conjunction with this qualitative analysis, alternative explanations for the genesis of stem cavities are also explored, including the hypothesis that the reallocation of carbohydrates within broccoli is responsible for the development of this disorder.

METHODOLOGY

Other studies have demonstrated that planting density influences the development of hollow stem in broccoli and cauliflower (Gorski and Armstrong, 1985, Zink and Akana, 1951, Hipp, 1973, Cutcliffe, 1971) and can be used to manipulate the levels of this disorder encountered (Everaarts and Putter, 2003). Given this, hollow stem development was studied in a trial consisting of two plant stand densities, commercial density (CD) and high density (HD), arranged in a complete randomised design with three replicates. The trial was conducted at the Forthside Vegetable Research and Development Station on the north west coast of Tasmania on a deep red earth (Ferrosol). Broccoli seedlings at the 4-5 leaf stage were transplanted on the 31st January 2007 at a commercial density of 32, 550 plants ha^{-1} (CD) and at a high density of 150, 000 plants ha^{-1} (HD). Plants were placed in two rows centred on the bed 400mm apart under CD and three rows centred on the bed 133 mm apart under HD. Plots were 10.2 m long across three beds delineated by 1.64 m tractor tyre widths (10.2 m x 4.92 m). All plots received 105 kg N ha^{-1}, 120 kg P ha^{-1} and 82.5 kg K ha^{-1} prior to planting. A further two applications of 53 kg N as urea were made at 42 and 64 DAP. Boron was present in the soil in an adequate level of 0.8 mg kg^{-1} (CSBP Laboratories, Kwinana, Western Australia).

HOLLOW STEM AETIOLOGY

The morphological stage of inflorescence development (modified from Tan et al., 1998; see Chapter 5) was monitored using the shoot apices of plants in the buffer rows of each planting density. Destructive sampling of plants from each plot commenced at 37 DAP when the majority of plants were either transitioning towards or had commenced floral initiation. Two plants from each plot were removed at 3-4 day intervals and assessed for stem height (mm), widest stem width (mm), presence or absence of a stem cavity and the morphological inflorescence stage. The presence of a stem cavity was defined as any split or larger fissure in the stem pith tissue that was visible to the naked eye. Leaves were then removed and the stem sectioned into two node intervals from node 5 upwards until node compression at the tip of the stem made further divisions impractical. Node 5 was the lowest node sectioned as below this the stem tissue is typically woody and hard to dissect. Each section was then photographed using a digital camera (Powershot G5, Canon Inc.) and a core sample removed as described below. The remaining plant tissue was then dried in an oven set at 70 °C and weighed. A final harvest was made at 87 DAP when the average inflorescence in the CD treatment was still compact in appearance, 100-200 mm in diameter, and the flower bud size was less than 3mm. The description of hollow stem made here was also supplemented from observations made of this disorder in other plants grown at 19, 512 and 32, 520 plants ha^{-1} in the trials outlined in Chapter 8.

STARCH DETERMINATION

From each 2 node section of stem a 9 mm diameter longitudinal core of the central pith tissue taken using a cork borer. The core sample was immediately placed in a scintillation vial and frozen in liquid nitrogen. Samples were then stored at -80.0 °C and later dehydrated in a freeze dryer (Heto Drywinner, Model DW 1, 0-110, Denmark) until the dry weight curve reached its asymptote. Sampling of the stem

tissue continued up to 62 DAP at which time the development of hollow stem prevented further sampling in the CD treatment.

Dehydrated samples were then assayed for starch using an enzymic Total Starch Determination kit (Megazyme Pty Ltd, Australia). Sub samples of the stem core cores weighing 100 mg were placed in a 20 mL glass test tube and partially hydrolysed and fully solubilised by first adding 3 mL of thermostable α-amylase (50 mM, pH 7.0). The solution was mixed with a vortex mixer (Chiltern Scientific, Australia) and then incubated in water at 100 °C for 6 minutes while mixing was repeated at 2, 4 and 6 minutes. Starch dextrins were then quantitatively hydrolysed to D-glucose by placing the tube in water at 50 °C, adding 4 mL sodium acetate buffer (200 mM, pH 4.5), and then adding 0.1 mL amyloglucosidase. Each sample tube was then vortexed and incubated for a further 30min at 50 °C. Samples were then adjusted to 10 mL, vortexed and centrifuged at 3, 000 rpm for 10 minutes. Three (3) mL of the supplied GOPOD reagent (glucose oxidase 12 , 000 units L^{-1}; peroxidise > 650 units L^{-1}; 4-aminoantipyrine 80mg) were added to 2 x 0.1 mL aliquots and incubated for a further 20 minutes at 50 °C. After incubation the absorbance at 50 nm for each sample was determined and cross checked with the D-glucose control and reagent blanks. Starch content was calculated in mg kg^{-1} using:

$$starch = \frac{\Delta E \times F}{W} \times 90$$

where ΔE is change is absorbance when compared to the reagent blank, F is 100 / absorbance for 100 µg of glucose, and W is the sample weight.

ESEM

Broccoli stems were collected from a commercial crop at West Pine (north west Tasmania) on the 29th April 2008 during early head development. To minimise water loss from the stem representative plants were carefully excavated from the soil and defoliated using a sharp boning knife. The stem was placed in a sealed

plastic bag with the root ball remaining intact. The bagged stems were then kept on ice overnight before being prepared for examination under a Quanta 600 MLA (FEI Company, Oregon,USA) environmental scanning electron microscope (ESEM). For each stem collected the head was removed and the pith examined for stem cavities. Samples of stem cavities from fine initial fractures to tissues from the surfaces of the elliptical secondary cavities (described later) were selected for examination. The pith tissue of interest was dissected from the stem, mounted as a fresh specimen and observed under low vacuum at magnifications ranging from 500x to 800x (10 – 15 kv, 5.6 Torr, 5 oC).

RESULTS

HOLLOW STEM AETIOLOGY

Hollow stem was first observed in the CD treatment at 49 DAP during late inflorescence development (Stage 6 (Chapter 5); Figure 1) just prior to buttoning. In the plants first affected by hollow stem, the cavities first appeared in the upper most part of the stem. All CD plants had developed hollow stem by harvest, with an average 62 ± 13 (SEM) % of nodes being affected on this date. The cavities in all CD plants at harvest extended from approximately node 10 upwards and into the inflorescence pith tissue. Brown tissue surrounding the cavity first appeared 65 DAP. Plants grown under HD reached buttoning (Stage 8) *ca.* 10 days after CD treated plants and did not develop hollow stem, internal tissue browning or a head of suitable size for commercial harvest.

When viewed as a transverse section of stem, cavity development began as a thin, linear (Figure 2A) or crescent shaped (Figure 2B) split in the central pith, just below the inflorescence but still below the compressed upper nodes (Figure 1).

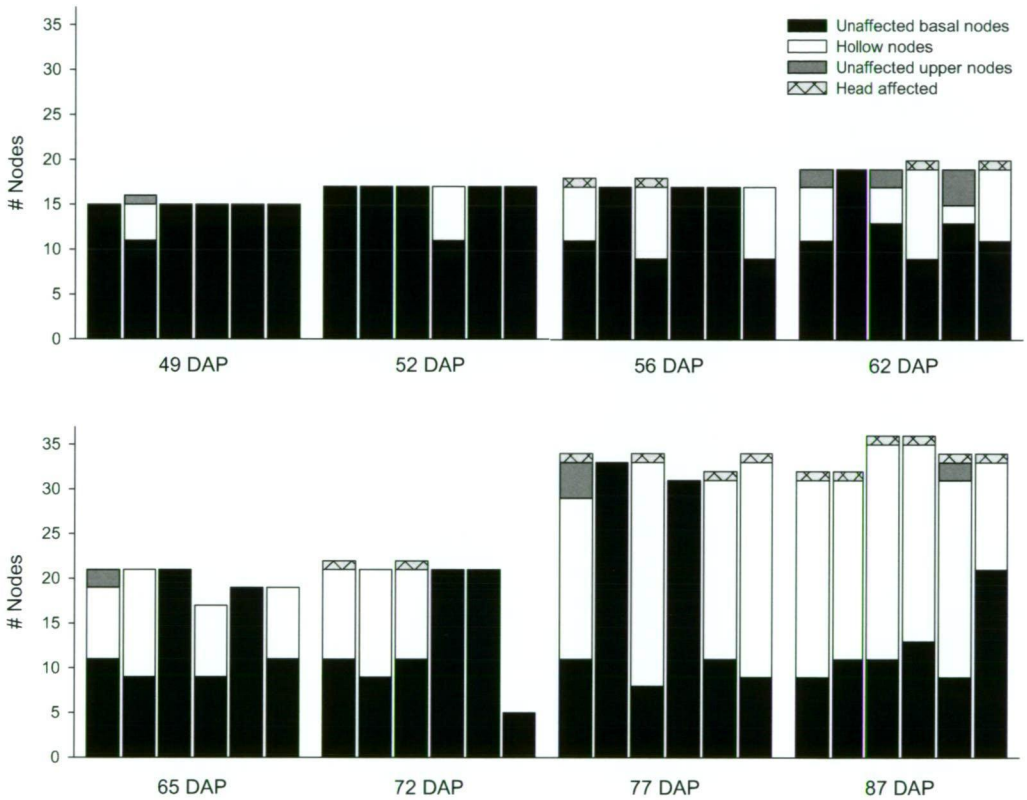


Figure 1. *Hollow stem development from 49 to 87 DAP under the commercial density treatment; indicating the time of development and position within each plant that cavity formation occurred. Each of the six plants harvested on each date is represented by a bar. The hollowed nodes and their position within the stem are indicated by the clear section of the bar (□). All plants had heads from day 56 onwards, however only heads affected by hollow stem are indicated in the graph.*

The initial split either occurred on the edge of, or dissected the central pith cylinder tissue, which was often 'watery' in appearance. While also propagating longitudinally throughout the stem with time, the splits widened perpendicular to its main axis in the transverse plane and also extended radially towards its perimeter. Radial propagation of the stem appears to be restricted to the pith tissue, however in well developed cavities propagation could extend to just inside

the vascular tissue (Figure 2D) with the cavity then consuming almost the entire region. The fractures in some stems also began to branch as radial propagation progressed, often becoming trigonal in nature when viewed as a transverse section (Figure 2C). Most longitudinal propagation of the cavities occurred in the upper section of the stem (Figure 1) where inter-nodal elongation was occurring.

As cavity development progressed, the walls perpendicular to the major radial axis of the primary cavity developed secondary cavities that were elliptical in shape. When viewed in cross section, these secondary cavities were triangular in shape. The major axes of these elliptical cavities were perpendicular to that of the primary cavity. Longitudinal fracturing in the wall of the primary cavity was also observed between secondary cavities (Figure 2D).

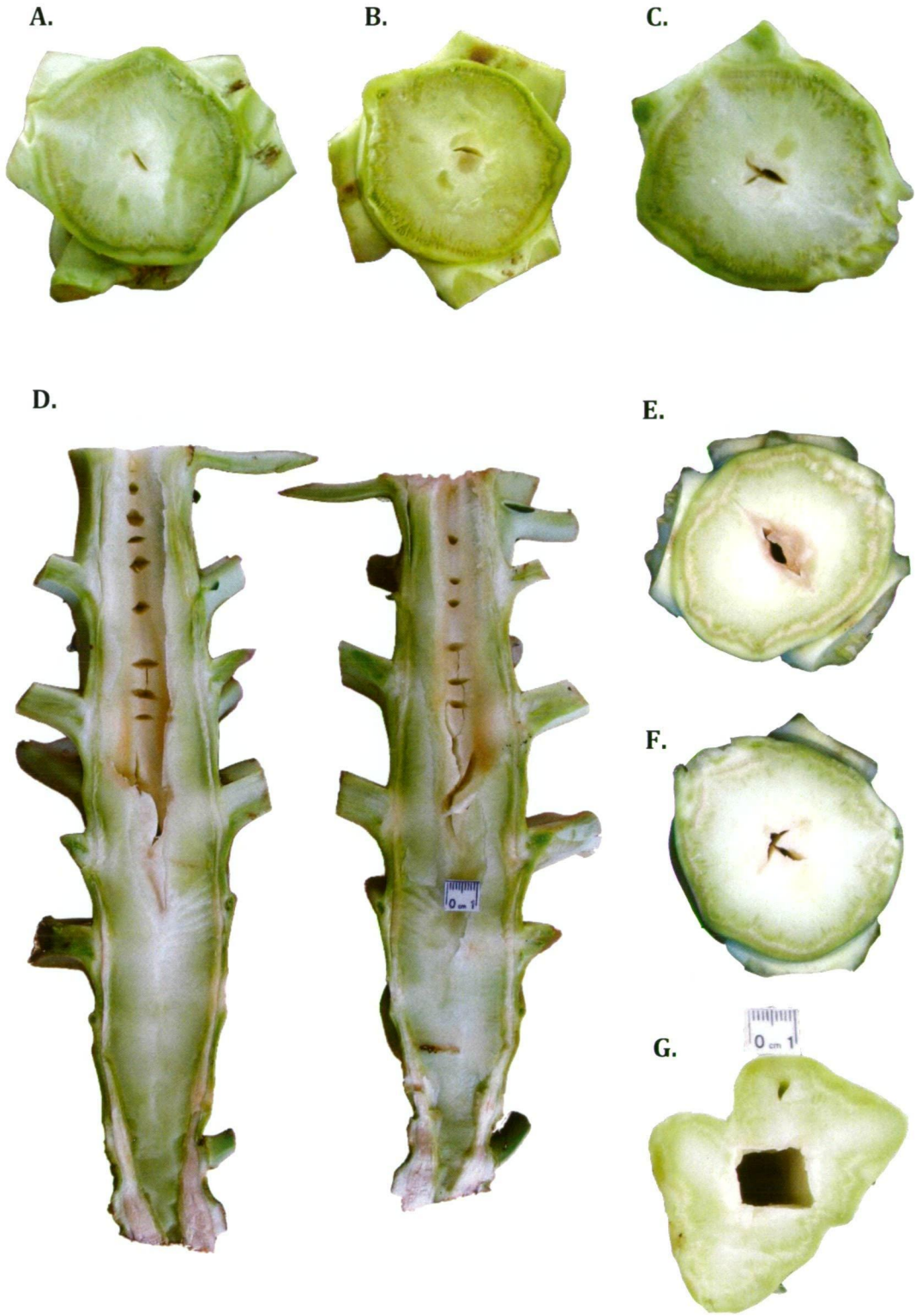


Figure 2. *Initial hollow stem development* ***(A-C)*** *and hollow stem in less well developed* ***(E,F)*** *and well developed cavities* ***(D,G)*** *at harvest.*

The cavities in advanced hollow stem were of uniform dimensions throughout the middle and top of the stem, but often tapered within the inflorescence, and at the base of the stem, became an irregular fissure (Figure 2D). Well developed cavities were often smooth walled and were nearly always star shaped in cross section (Figure 2G). Less well developed cavities were observed to change in cross sectional shape throughout the stem and could be elliptical, trigonal or star shaped in cross section (Figure 2E & F). Some plants at harvest were observed to have what appeared to be two independent cavities, one large cavity transgressing the middle of the stem, and a second small cavity in the inflorescence pith (Figure 1).

Within the inflorescence pith, the cavities had a sculpted appearance if the stem was dissected longitudinally through the fracture axis of the primary cavity (Figure 3A). Secondary elliptical cavities were also present in the walls of the inflorescence pith tissue. In the majority of cases the cavity shallowed as it tapered into a dome

Figure 3. *Hollow stem in broccoli inflorescence.* ***(A)*** *Inflorescence bisected longitudinally and parallel to axis of the main cavity.* ***(B)*** *An inflorescence bisected longitudinally and perpendicular to axis of main cavity.*

shaped peak when viewed from this angle When the head was sectioned longitudinally but perpendicular to the axis of the main cavity, the walls met in an acute apex with evidence of tissue tearing often being evident (Figure 3B). The top of the cavity also met in an acute apex when viewed from this perspective. In some plants with severe cavities, the hollow continued through the peak of the inflorescence stem, leaving it open to the environment.

Tissue necrosis accompanied most but not all instances of hollow stem, and in some cases extensive necrosis was observed in the absence of cavities. When present within the cavities, the brown discolouration was either light (Figure 4C), formed a distinct halo of brown tissue in the cavity walls (Figure 4A), or

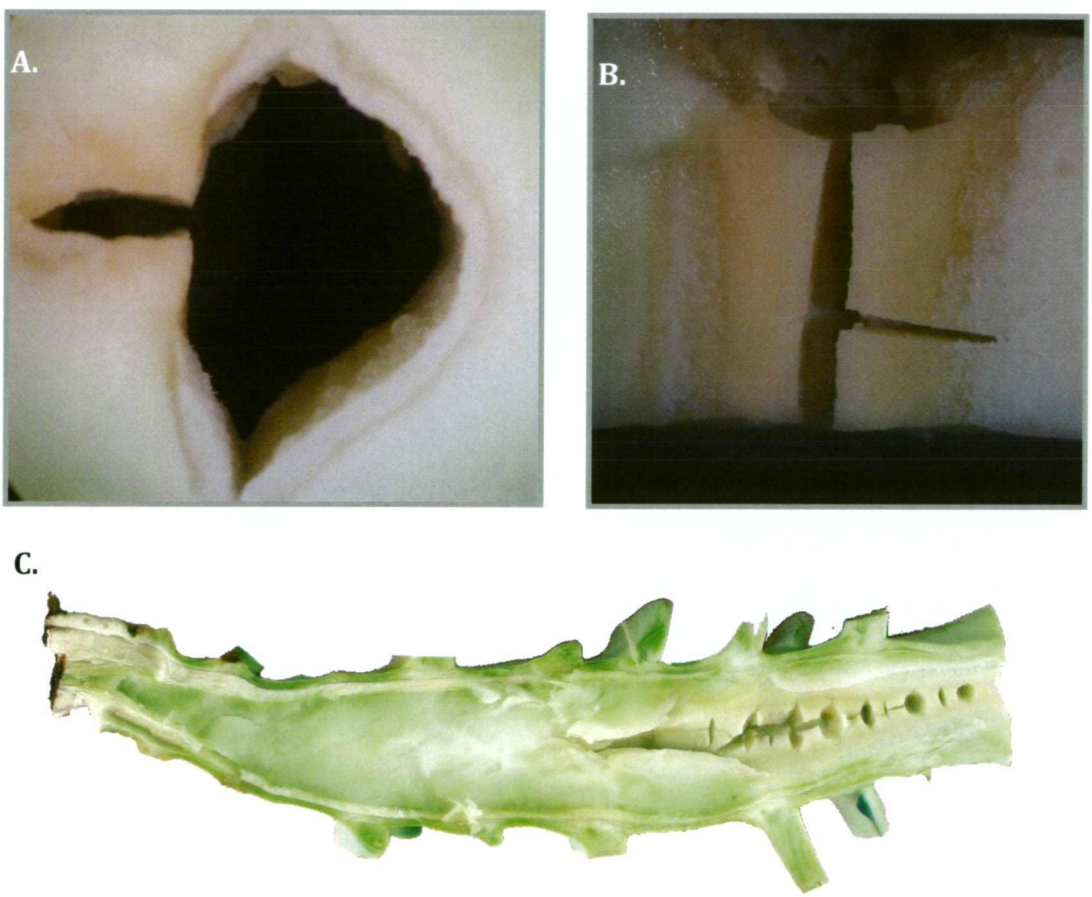


Figure 4. *Browning of stem pith tissue associated with the formation of stem cavities.* ***(A)*** *Central pith tissue heavily affected by tissue browning.* ***(B)*** *Cavity with a 'halo' of brown tissue forming in the outer tissue of the stem wall.* ***(C)*** *Stem with an extensive cavity only exhibiting light browning of the tissue.*

extensively affected the central pith cylinder (Figure 4B). Light necrosis seemed to be restricted to very most outer layer of the tissues comprising the cavity wall and was not associated with severity as it was often found with extensive cavities as well as those less well developed. In stems with heavy browning, the tissue in the centre of the pith appeared brittle and of a different texture to the surrounding tissue (Figure 4B). This type of tissue discolouration was again not associated with severity, and could be seen in both minor (Figure 2E) and extensive cavities (Figure 4C).

BORON DEFICIENCY SYMPTOMS

Apart from cavity development and the associated tissue necrosis, the plants that developed hollow stem under CD did not exhibit many of the symptoms reported to accompany a boron deficiency. The leaves of these plants did not appear to be brittle or stunted, did not have callused veins or exhibit any signs of rolling or epinasty. There were no signs of oedema on the petiole or longitudinal cracking of the epidermis, and the inflorescence's appeared normal, with no floret abortion or shape deformation (Benson et al., 1961, Chandler, 1940, Shelp, 1990, Shelp et al., 1992). The oldest leaves did yellow and senesce, particularly at the HD, however this is likely the result of nitrogen flux within the plant. There was no evidence of leaf margin browning (Gupta and Cutcliffe, 1975).

ESEM OF HOLLOW STEM CAVITIES

The tissues observed in this study indicate that both cell separation and fracture were involved in the development of stem cavities. Central pith tissue with newly developed fractures revealed intact cells in the cavity walls perpendicular to the transverse axis of the fissure (Figure 5A). This suggests that the widening of the fissure perpendicular to its axis involved cell separation. Tissue sampled from the front of the radial propagation (Figure 5B) revealed signs of tearing and cell wall deformation. It was unclear from the images as to whether this was cell wall separation or mechanical destruction of the cell walls. Cellular debris was

observed and this would suggest that that some cells were being destroyed during radial propagation of the fissure. Pith tissue sampled from the transverse face of a secondary elliptical cavity showed clear signs of cell fracture (Figure 5C). The tissue in Figure 5C exhibited surface browning.

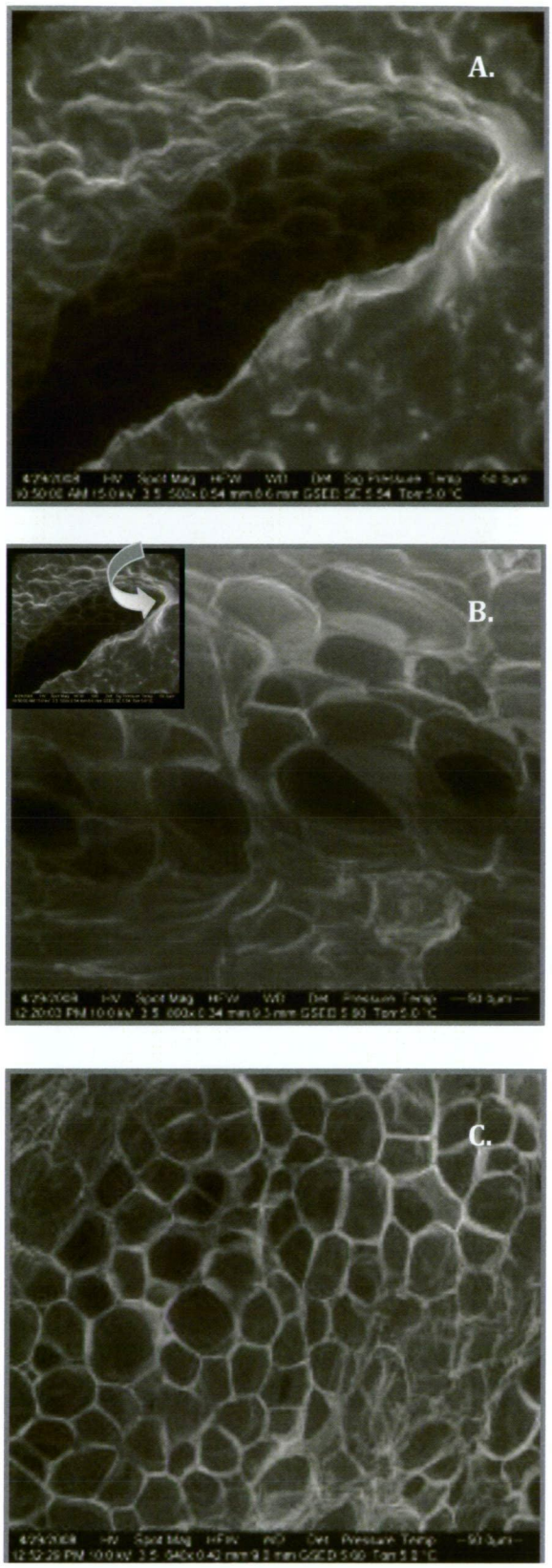


Figure 5. *ESEM imagery of hollow stem affected tissue.* ***(A)*** *Transverse section of stem tissue showing a newly initiated fracture.* ***(B)*** *Tissue at the radial front of fracture propagation (see inset) showing cell separation and cellular debris.* ***(C)*** *Tissue sampled from the transverse face of a secondary elliptical cavity showing cell fracture.*

STARCH

Stem starch levels in the central pith declined during inflorescence initiation and curd development under both planting densities. The levels of starch were lower under CD on all 5 sampling dates. A general decrease in starch levels was recorded over the sampling period for plants grown at HD, while plants grown at CD exhibited a decline in stem starch level from head initiation (37 DAP) until about the first sign of hollow stem (Figure 6). The experimental design did not allow for a direct comparison of starch between those plants free and affected by hollow stem, however all plants at the lowest density eventually developed stem cavities, and no cavities developed at HD. There did not appear to be any pattern in starch levels across nodes on a particular date or within nodes across dates (data not shown).

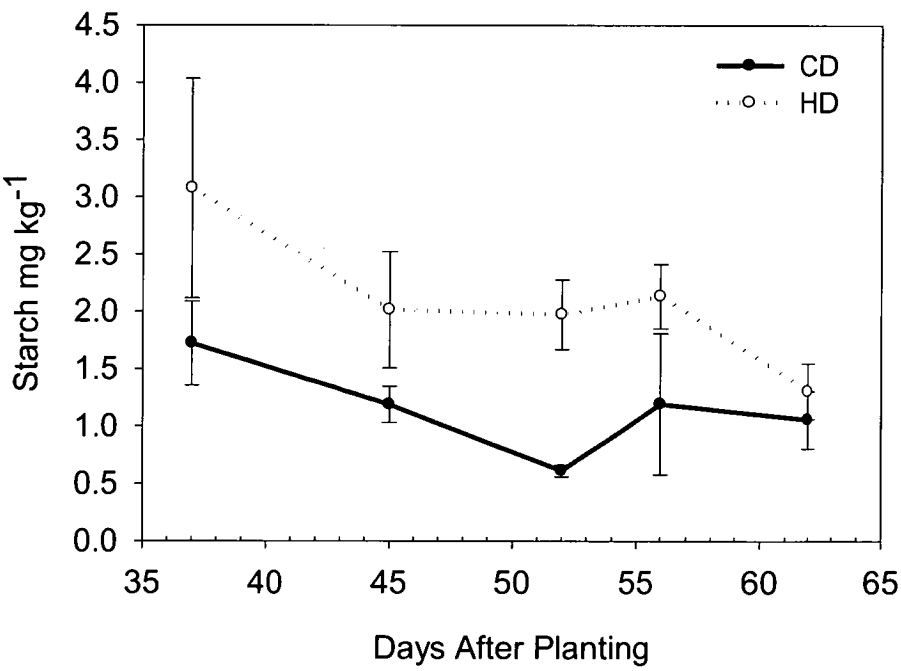


Figure 6. *Starch levels in the central pith tissue of broccoli stems grown at CD (32 550 plants ha^{-1}) and HD (150 000 plants ha^{-1}). Error bars are SEM.*

EFFECT OF DENSITY ON PLANT GROWTH

The plants grown under CD were not stunted and produced heads equal in quality and size expected for a commercial harvest. The plants grown under HD however did not produce a marketable inflorescence. Dry weight accumulation of the whole plant during head development continued to increase until harvest (Figure 7). Similarly, middle (widest) stem width increased linearly until harvest throughout inflorescence development stage at both densities; however the rate of stem widening was greater at the commercial density (CD) planting. Stem height also continued to increase throughout the life of the crop, proceeding at a greater rate under the high density (HD) planting. Plants grown at CD were therefore heavier, with shorter and wider stems than the plants grown at HD under which plants accumulated much less dry matter and had thinner but somewhat taller stems.

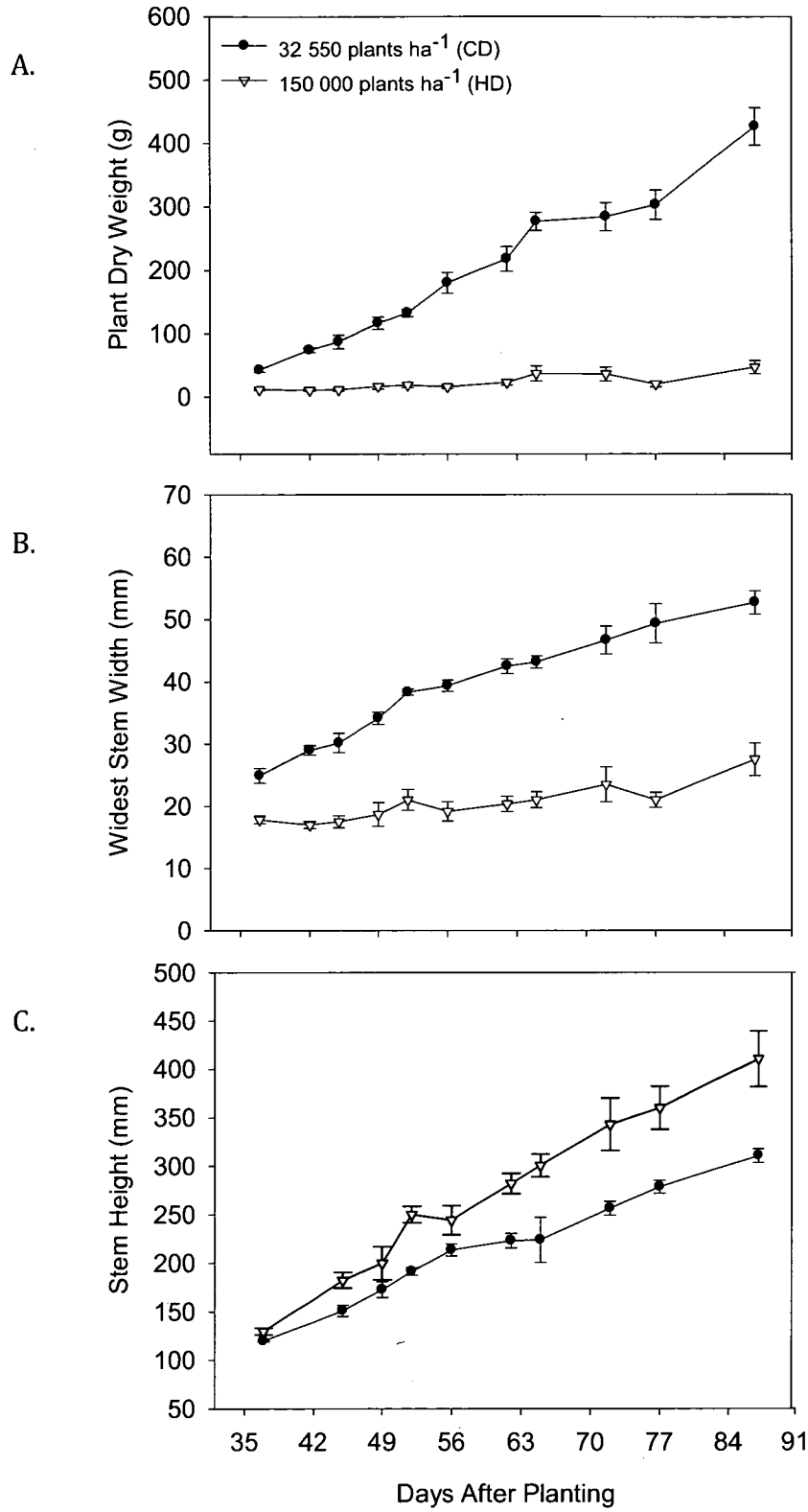


Figure 7. *Broccoli growth rate and morphology at two densities, CD (• 32, 550 plants ha^{-1}) and HD (▽150, 00o plants ha^{-1}), expressed as* ***(A)*** *dry weight,* ***(B)*** *widest stem with and* ***(C)*** *stem height 37 to 91 days after planting. Error bars are SEM.*

DISCUSSION

The etiology described here is more detailed than that previously described and provides some evidence to suggest that mechanisms other than boron deficiency may be involved in the genesis of hollow stem. The symptoms described were similar to that previously described for hollow stem in other studies however were generally not consistent with that described for boron deficiency.

In this study hollow stem did initiate in the top of the stem, just below the newly initiated inflorescence as observed in previous studies (Zink, 1968, Cutcliffe, 1972). The consistency with which this occurs suggests that the development of hollow stem is intimately linked with inflorescence initiation. However, because this does not occur in all plants, or in all crops, it would appear that cavity development at this growth stage is mitigated by additional factors. While other researchers have reported that hollow stem often occurs in the absence of tissue necrosis, in this study it was not so, with most stems exhibiting some degree of tissue browning (Cutcliffe, 1972, Zink and Akana, 1951, Shattuck and Shelp, 1987). This was not always the case, and as the discolouration was often quite light with some stems not showing any discolouration at all, appears to be relatively consistent with previous reports in the literature. Previous work has also described hollow stem as beginning with transverse elliptical cavities (Shattuck and Shelp, 1987, Zink and Akana, 1951, Cutcliffe, 1972) yet here the development of elliptical cavities in the transverse plane were observed subsequent to an initial longitudinal crack as described by others (Griffith and Carling, 1991). As the stems in this research were dissected through the transverse plane during development and only dissected longitudinally at harvest, it is possible that small elliptical cavities were present at the time initial longitudinal fractures were first observed.

The development of stem cavities and the appearance of necrotic tissue with, and sometimes without cavities, is consistent with a boron deficiency. The appearance of the initial fracture in broccoli was often preceded by a central cylinder of tissue in the pith becoming water soaked in appearance, and this is also consistent with the symptoms described for boron deficiency. The appearance of water soaked tissue in the centre of the stem has also been described in association with this

disorder in cauliflower (Dearborn, 1942). In this subspecies, stem cavity development under a boron deficiency started primarily in the parenchyma of the pith, but also in the cortex tissues. Beginning with the enlargement of isolated parenchyma cells, intracellular spaces were reduced in size and then observed to fill with a substance of "mucilaginous or gummy" appearance. This region of tissue subsequently turned brown in colour, staining positive for lignin. After this a fine transverse split propagated though the intracellular space, suggesting that cell separation was occurring. This description of the initial stem fracture in cauliflower is markedly similar to that observed for broccoli in this study.

Despite these similarities, many other symptoms associated with a boron deficiency in broccoli such as stunted growth or death of the apical meristem; oedema's or corky lesions on the petioles and stem; leaf brittleness, epinasty, or rolling; nor misshapen heads with aborted florets, were not observed (Shattuck and Shelp, 1987, Chandler, 1940, Benson et al., 1961, Shelp et al., 1992). In addition to this, some of these symptoms, such as leaf rolling and epinasty, seem to appear prior to the development of stem cavities (Chandler, 1940). Soil boron levels were also adequate, and under the HD treatment, under which competition for soil nutrients would have been greatest, no stem cavities were observed. While some of the symptoms observed were similar in nature to boron deficiency, overall the two disorders seem to be readily distinguished from each other, in the case of hollow stem, by the absence of the additional symptoms associated with boron deficiency.

Stem cavities did not develop in plants grown at the highest planting density, and as with other studies (Griffith and Carling, 1991, Everaarts and Putter, 2003, Cutcliffe, 1972, Zink and Akana, 1951), further illustrates the relationship between hollow stem and some aspect of plant stand density. The foremost effect of plant stand density was on growth rate, with the highest planting density causing a dramatic reduction in plant dry weight. Plants under the high density treatment also had an altered architecture, with these plants having thinner, taller stems with no cavities. This data supports earlier assertions that plant size or growth rate may be linked to the development of hollow stem (Griffith and Carling, 1991, Zink and Akana, 1951).

The association between hollow stem and growth rate, and its appearance at the time of inflorescence initiation, suggests that carbohydrate reallocation as one possible mechanism underlying the genesis of stem cavities. The appearance of water soaked tissue in the central stem pith is also similar in nature to watercore of apples, and this latter disorder is also thought to be associated with the mobilisation of carbohydrates (Faust et al., 1969, Yamada et al., 2006). The development of the broccoli inflorescence is rapid, with this organ proceeding from a small button approximately 10mm in size, to a fully fledged head 150-200 mm in diameter in a relatively short period of time. Rapid growth of the head most likely places a high demand for assimilates supplied via photosynthesis and other metabolic processes. This demand may not be met, and the shortfall in assimilates may in turn cause the plant to remobilize carbohydrates stored in the pith tissue as starch (Carr and Jaffe, 1995). This hypothesis is to some extent supported by the observed decline in the central pith starch levels that occurred during inflorescence initiation. The decline, which occurred at both densities, could be interpreted as evidence of early preparation for pith autolysis, yet, stem cavities only occurred at the CD. The lower starch levels observed at the CD may be related to the difference in cavity development, and it is interesting that the level of starch within these stems did not drop further after stem cavities were initiated.

The presence of cell separation in the initial longitudinal split and the cell fracturing in the secondary elliptical cavities has not been reported previously. This type of tissue separation, particularly the cell wall fracturing, is indicative of mechanical stress within the pith tissues. The tearing of tissues via cell fracture in response to stressed induced by uneven growth of anisotropic tissues has been reported for carrots (McGarry, 1993, Gracie and Brown, 2004), and it may be that a similar mechanism underlies the origin of stem cavities in broccoli. The secondary elliptical cavities were invariably located in the wall of the main cavity, perpendicular to its major radial transverse axis, and might also be symptomatic of mechanical tissue stress. The major radial axis in large cavities typically extended almost to the vascular tissue of the stem. In stems with this type of severe cavity, there is little capacity for this tissue to develop a secondary elliptical cavity due to the thinness of the pith wall. The perpendicular orientation of these secondary

cavities, typically centered on the minor transverse radial axis of the main cavity, is therefore possibly indicative of longitudinal tissue stress developed by inter-nodal elongation on the thicker pith tissues of the main cavity wall. As node production below the leaf node ceases at inflorescence initiation (Tan et al., 1998), the continued increase in stem height during development of this organ must have been due to inter-nodal elongation, and thus it is possible that this process places stress on tissues that might otherwise in an intact stem, be under compression (Hejnowicz, 1997). The development of mechanical stress within the pith tissues may also be related to stem width (Griffith and Carling, 1991) and this observation is supported by other studies that have noted the link between stem size, rapid growth and hollow stem in broccoli (Zink and Akana, 1951).

In this study we have improved on the published description of hollow stem and shown that it is significantly different from previous descriptions related to boron deficiency, and while stem cavity formation is a common symptom, it is plausible that these are two distinct disorders. The described symptoms and the influence of plant density also leave open the possibility that hollow stem development is a function of plant growth rate or size. The data relating to starch did not preclude carbohydrate reallocation as a mechanism involved in the development of hollow stem, and the effect of density might be related to such a mechanism. The observation of cell separation, fracture and tissue tearing also suggests that mechanical tissue stress may be an additional or alternative mechanism involved in the development of this disorder.

CHAPTER 8

GROWTH RATE AND THE DEVELOPMENT OF HOLLOW STEM IN BROCCOLI

INTRODUCTION

The formation of stem cavities in mature broccoli (*Brassica oleracea* L. var. *italica* Plenck) and cauliflower (*Brassica oleracea* L. var. *botrytis* DC.) heads is a significant issue for both fresh market and processed produce. Commonly referred to as hollow stem, the disorder is often accompanied by browning of the affected tissue and may allow the ingress of pathogens or soil. Heads with hollow stem are therefore unattractive to consumers and prevent processors from dicing the stems for use in frozen products. The disorder may cause significant financial losses as the incidence of hollow stem in a crop can be as high as 90%.

Identification of causal factors has been the focus of research on the disorder over the past 75 years. Hollow stem in broccoli and cauliflower has been attributed to both boron deficiency (Chupp and Horsfall 1933; Dearborn and Raleigh 1935; Shattuck and Shelp 1987; Shelp 1988; Shelp et al. 1992) and to factors linked to growth rate such as high nitrogen application (Zink 1968; Cutcliffe 1972; Hipp 1973; Vigier and Cutcliffe 1984; Gorski and Armstrong 1985; Tremblay 1989; Belec et al. 2001), planting density (Cutcliffe 1972; Cutliffe 1975; Gorski & Armstrong 1985; Griffith & Carling 1991; Zink 1968; Zink & Akana 1951) plant size (Nieuwhof 1969; Scaife and Wurr 1990), and irrigation (Babik and Elkner 2002; San Bautista et al. 2005). Even though both mechanisms have been reported in the scientific literature, boron deficiency has been widely accepted by industry as the dominant cause. Thus application of boron is the most common commercial recommendation found in the popular literature to prevent the development of hollow stem. Contrary to this recommendation, the occurrence of stem cavities in broccoli has been reported in instances where soil or tissue boron levels are

considered adequate (Tremblay 1989; Everaarts and Putter 2003) while other studies have reported no association between soil or plant tissue boron levels and cavity formation (Zink 1968; Gupta and Cutcliffe 1973; Hipp 1973; Gupta and Cutcliffe 1975; Vigier and Cutcliffe 1984; Scaife and Wurr 1990; Griffith and Carling 1991).

Descriptions from field research studies of hollow stem report a characteristic set of symptoms. In the majority of studies of the disorder, hollow stem has simply been recorded as the presence or absence of cavities (Zink and Akana 1951; Gupta and Cutcliffe 1973; Hipp 1973; Gorski and Armstrong 1985; Peck and MacDonald 1986; Shattuck et al. 1986; Griffith and Carling 1991; Perniola et al. 1993; Coulombe et al. 1999; Belec et al. 2001; Brainard and Bellinder 2004; San Bautista et al. 2005). In a smaller number of studies the disorder is described as beginning in the centre of the stem soon after initiation of and just below the terminal inflorescence, with elliptical transverse gaps that progressively enlarge to form interconnected cavities extending longitudinally throughout the stem. The cavities do not necessarily include necrotic tissue, although this may develop after harvest (Zink and Akana 1951; Cutcliffe 1972; Hipp 1973; Wyatt et al. 1989). Additional symptoms beyond cavity formation were not mentioned in these studies, although Zink (1951) specifically noted the absence of bud deterioration associated with boron deficiency despite the occurrence of hollow stem in sprouting broccoli cultivars.

In contrast to the simple set of symptoms described for hollow stem in field studies the symptoms associated with imposed boron deficiencies, notably from glasshouse studies, are quite extensive, and in addition to stem cavities include; leaf rolling, epinasty, cracking of the lamina and petiole, callus formation on leaf veins, leaf browning and senescence, bud deterioration and necrosis of stem tissue (Chandler 1940; Chandler 1941; Benson et al. 1961; Gupta and Cutcliffe 1975; Shattuck and Shelp 1987; Shelp 1988; Shelp 1990).

While a number of studies have suggested growth rate, or factors affiliated with this, are linked to the development of hollow stem, there is only one study (Everaarts and Putter 2003) where direct evidence using calculated growth rates

has been presented. In the study by Everaarts and Putter (2003), growth rate in cauliflower was manipulated using a range of planting densities and the incidence of hollow stem increased concomitantly with absolute growth rate (g day^{-1} fresh weight) of the stem.

The hypothesis investigated in our studies was that high plant growth rate, rather than boron deficiency, induced hollow stem in broccoli.

MATERIALS AND METHODS

SITE AND CLIMATIC CONDITIONS

The trials were conducted at the Forthside Vegetable Research and Development Station (E 438236 N 5438407; GDA94) situated at Forth on the North West coast of Tasmania, Australia. Tasmania has a temperate maritime climate, comprised of cool dry summers and a predominately wet winter. Forth's mean total annual rainfall is 1010 mm, minimum average temperature 6 °C and maximum average temperature 15 °C. The research station, with elevations of 90-150 m above mean sea level, is situated on a red ferrosol soil type, a deep friable clay loam derived from basaltic parent material.

TRANSPLANTING

Broccoli seedlings (cv. Marathon) were produced commercially in 15 cm^3 cell trays (Lannen Plant Systems L256) and transplanted into cultivated soil. Seedlings were placed in two rows 400 mm apart on beds delineated by 1.64 m tractor wheel centres. Fertiliser was banded approximately 100 mm below each row of transplants in 200 mm bands.

EXPERIMENT 1

Seedlings were transplanted on 30th September 2005. Five hundred and seventy kg ha^{-1} of di-ammonium phosphate (103 kg N ha^{-1}; 114 kg P ha^{-1}; 6 kg S ha^{-1}) was incorporated prior to planting. Hot water soluble soil B was assayed at 1 mg kg^{-1} (CSBP Ltd. Laboratories, Kwinana, Western Australia) and no additional boron was incorporated at planting. Treatments were allocated using a split plot design. Main plots (4.8 m x 16 m) were assigned in 3 blocks, and either supplemented with a foliar boron solution (+B) or left untreated (-B). Each main plot was further divided into 3 randomly allocated planting densities; High Growth (HG) at 19, 512 plants ha^{-1} + 95 kg N ha^{-1}; Commercial Growth (CG1) at 32, 520 plants ha^{-1} + 95 kg N ha^{-1}; and Low Growth (LG) at 69, 686 plants ha^{-1} with no additional N. The additional top dressing of urea was applied to the HG and CG1 plots on 24th November 2005. Split plots were demarcated by the bed system at 1.6 m x 16 m. The planting densities were employed to manipulate growth rate (Everaarts and Putter 2003).

Two Solubor DF (Borax Inc.) applications were made at 2 kg ha^{-1} (0.35 kg B ha^{-1}; pH = 8.22) on the 16 November 2005 and 1st December 2005 to the +B treatment plots during inflorescence initiation and early buttoning stages of crop development respectively.

Three plants were randomly selected from each subplot and harvested when the heads were still compact, approximately 150 mm in diameter and with flower buds less than 3 mm in diameter. Roots were severed from the main stem at the cotyledon scar and the inflorescence was severed at a distance of 140 mm from its dorsal surface. Heads with branches below 140 mm were cut on the underside of the insertion point of the lowest branch. Digital photographs (Power Shot G5, Canon Inc.) were taken of the dorsal surface of the head to later establish the equivalent head diameter using image analysis tools. To enable accurate measurement a scale marker was placed on the horizon of the head skirt, the plane in which head diameter was taken. Both the head and stem of each plant were then longitudinally dissected and digital images taken of each half to enable measurement of the length of each organ, and the widest point of the stem. Heads

were bisected longitudinally, cutting radially through the lowest inflorescence branch (II order, position 1 branch). Scale markers were placed at the face of the cut surface for each half of both heads and stems. The presence or absence of hollow stem was recorded and severity later estimated using image analysis. Each plant was separated into stem and petiole, the 6 youngest leaf laminae, the middle leaf laminae, the 6 oldest leaf laminae, and the inflorescence. The fresh weight of each fraction was recorded, oven dried at 60°C and then reweighed.

EXPERIMENT 2

Broccoli seedlings were transplanted on 9th January 2007. All plots received 105 kg N ha^{-1}, 120 kg P ha^{-1} and 82.5 kg K ha^{-1} prior to planting. Boron was present in the soil at 0.8 mg kg^{-1} (CSBP Ltd. Laboratories, Kwinana, Western Australia) and not supplemented at planting.

A 2 x 4 factorial design comprised of 2 planting densities and 4 treatments randomised across 4 blocks was implemented. Planting densities were allocated at 32, 520 plants ha^{-1} (Commercial growth rate; CG2) and 100, 000 plants ha^{-1} rate (Very Low growth rate; VLG). Development of the plant meristems was monitored in guard row plants (via excision of the shoot tip) and treatments were applied during inflorescence stage 6 (Tan et al., 1998) (36-42 DAP) just prior to the postulated appearance of hollow stem.

Treatments were comprised of a foliar trace element application applied at label rates to mimic commercial application (Ferti-mix; Campbell's Fertilisers Australia), shading (57% interception), Paclobutrazol (Gibberellin synthesis inhibiting growth regulant; Condense, Nufarm Australia Ltd.) and an untreated control (UT). Foliar fertiliser (FF) (1.5% B) was applied at 0.75 kg ha^{-1} in 1 litre of water per plot on 3 occasions at 10-14 day intervals from 13th February 2007 onwards. Shading (SH) was applied on 20th February 2007 by suspending shade cloth between steel pickets past the edge of the plots. Only plants shaded all day were used for sampling from this point onwards. Paclobutrazol (PB) was applied to the leaves at

480 g a.i. ha^{-1} on the 14th February 2007 and washed into the soil 1.5 hours after application by irrigation. Each plot measured 4.6 m x 4.9 m.

One randomly selected plant per block from each treatment was destructively sampled at 14 day intervals until harvest to attain whole plant dry weights. Three plants from the centre of each plot were cut when harvest maturity was reached. Data collection and digital images taken in the same manner as the previous trial, however the leaves were not divided into age classes, nor were the lamina separated from the petiole. The widest point of the stem, measured using image analysis in Experiment 1, was measured physically using digital calipers in this trial. All remaining plants, with the exception of buffer row plants, were cut and examined for the presence or absence of hollow stem to calculate total plot hollow stem incidence.

DATA COLLECTION VIA IMAGE ANALYSIS

Various morphological traits were measured from digital images taken immediately after harvest. The images were prepared for analysis in Adobe Photoshop CS (Adobe Systems Inc.) and analysed using Fovea Pro 4.0 (Reindeer Graphics Inc) or Image J. Traits measured included average head diameter, head height, stem length, widest point of the stem (Experiment 1 only), hollow stem incidence and severity. All dimensions computed were calibrated using scale markers placed in the appropriate plane.

The equivalent head diameter (d) was calculated from the 2-dimensional surface area of the head

$$d = 2 \times \left(\frac{A}{\pi} \right)^{0.5}$$

where A = area and π, the ratio of a circles circumference to diameter. Height of the heads was measured from one half of the longitudinally dissected head, using the y dimension of a bounded rectangle extending from the dorsal surface of the head to its excision point.

The area of one half of the bisected stem and the proportion affected by hollow tissue was calculated and referred to as severity. The area of the head stem tissue (first order axis only) with cavities was calculated using the same procedure.

Stem height was calculated using the y dimension of a bounded rectangle. Maximum stem width in Experiment 1 was recorded using the same procedure, this time using the x dimension. This parameter was measured using vernier calipers in Experiment 2. Head and stem height were used to calculate total plant height.

GROWTH RATE CALCULATIONS

Absolute growth rate (**AGR**) was calculated as

$$AGR = \frac{(w_2 - w_1)}{(t_2 - t_1)}$$

where w_1 is the initial dry weight, w_2 the final dry weight and t_1 and t_2 the initial and final sampling times expressed as days after planting.

STATISTICAL ANALYSIS

All analyses were undertaken with SPSS (ver. 14). A split plot model was used for the ANOVA of data in the first trial. Data in the second trial were analysed by 2-way ANOVA as a blocked 2 x 4 factorial. Angular transformations of percentage data were performed where homogeneity of variance or normality assumptions were violated. Hollow stem severity data in the second trial were transformed using a square root function to meet the assumptions of the analysis.

The SPSS logistic regression procedure was used to determine the relationship between growth rate and the probability of hollow stem affecting more than 5% of the stem. The 5% severity cut off was chosen as this increased the number of null values providing a better model.

RESULTS

HOLLOW STEM INCIDENCE AND SEVERITY INCREASED WITH GROWTH RATE

To determine if the different plant densities used to manipulate growth rate were effective, absolute growth rates were calculated for each treatment. Absolute growth rates increased concomitantly with the LG (69 868 plants ha^{-1}), CG1 (32 520 plants ha^{-1}) and HG (19 512 plants ha^{-1}) treatments in Experiment 1 (Figure 1A) and the VLG (100 000 plants ha^{-1}) and CG2 (33 000 plants ha^{-1}) treatments in Experiment 2 (Figure 1B) indicating that the treatments were effective. Absolute growth rates measured at harvest for CG1 (3.14 ±0.14 (SEM) g day^{-1}) and CG2 (3.28 ±0.13 (SEM) g day^{-1}) were similar allowing broad comparisons between the two trials.

Hollow stem incidence and severity were reduced in both trials by the LG and VLG rates when compared with the CG1, CG2 and HG rates (Figure 3). Both the incidence and severity of stem cavitation was higher in the untreated commercial growth rates in Experiment 1 (CG1) than Experiment 2 (CG2). The application of PB also reduced the severity of hollow stem formation at the commercial growth rate, although the incidence of hollow stem was not significantly different from the UT plants. This was despite PB having no influence on the absolute growth rate of the plants (Figure 1B). The application of SH or FF did not reduce the incidence or severity of stem cavity formation (Figure3).

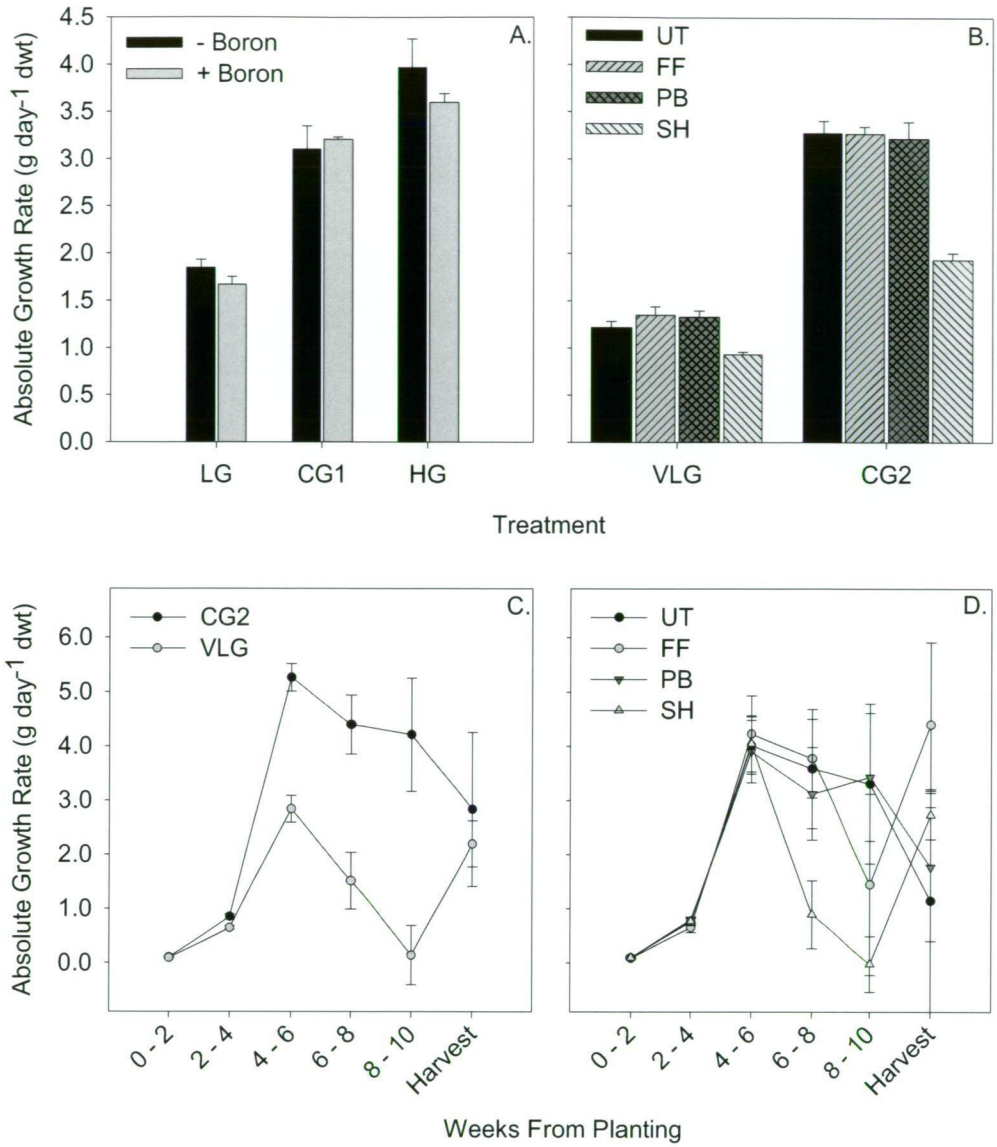


Figure 1. *Absolute growth rates (g day^{-1} dry weight) from Experiments 1 & 2. Experiment 1.* ***(A)*** *Boron application and growth rate treatment combinations; boron applied (+ Boron); no boron applied (-Boron) and HG (19 512 plants ha^{-1} + 198 kg N ha^{-1}), CG1 (32 520 plants ha^{-1} + 198 kg N ha^{-1}) and LG (69 868 plants ha^{-1} + 103 kg N ha^{-1}). Experiment 2.* ***(B)*** *Absolute growth rate calculated from transplant to harvest.* ***(C)*** *Absolute growth rate pooled by treatments at CG2 (33 000 plants ha^{-1}) and VLG (100 000 plants ha^{-1}) growth rates.* ***(D)*** *Pooled by density under the various growth rate treatments applied at inflorescence initiation, untreated (UT), foliar fertiliser (FF), Paclobutrazol (PB) and shade (SH). Error bars are SEM.*

TRACE ELEMENT NUTRITION DID NOT INFLUENCE HOLLOW STEM INCIDENCE OR SEVERITY

The application of boron alone as a foliar fertiliser (+B), or the application of a complete trace element mix (FF) containing boron did not significantly alter the

incidence or severity of hollow stem in either trial (Figure 3). Apart from stem hollowing and browning of the central pith tissues, no other symptoms previously reported for boron deficiency in broccoli, such as leaf epinasty, leaf rolling, leaf browning or yellowing, water soaked spots, floret abortion, callus formation on leaf veins, stem, lamina or mid rib cracking (Chandler 1940; Chandler 1941; Zink and Akana 1951; Benson et al. 1961; Gupta and Cutcliffe 1975; Shattuck and Shelp 1987; Shelp et al. 1992) were observed in either experiment. The application of Solubor produced a 4.6% increase in the concentration of boron from 28.1 ± 0.9 to 29.4 ± 0.6 ppm in the youngest leaf tissues in Experiment 1. There was no increase in leaf tissue boron between the untreated and FF plants in Experiment 2.

Extensive hollowing of the stem and head tissue was observed in both trials, and in the second experiment, browning of the central pith tissue, often without any significant hollowing, was also observed. The severity of stem tissue browning measured as the percentage of stem affected was not significantly different between the FF treatment and the UT plants or between growth rates (data not shown).

PROBABILITY OF HOLLOW STEM INCIDENCE INCREASED WITH GROWTH RATE

Despite the similarity in growth rates between treatments representing commercial planting regimes in experiment 1 and 2 (CG1 and CG2 respectively,) hollow stem severity and incidence in CG2 was approximately half that of CG1 (Figure 3). Both the manipulation of planting density and SH significantly ($P <$ 0.05) influenced final plant dry weights (Table's 1 & 3) and growth rate (Figure 1). Plant dry weight increased under CG1and HG treatments in Experiment 1 and CG2 in Experiment 2 (Table's 1 & 3). When compared to UT plants, SH reduced plant dry weight, but only at the CG2 rate. This effect was not apparent at VLG. The application of PB and FF in Experiment 2 did not reduce plant dry weight when compared to the UT plants.

When compared to the CG2 the VLG rate treatment reduced absolute growth rate over the 2 to 10 week period after planting in Experiment 2 (Figure 1C). A reduction in growth rate in response to SH at both densities was recorded after application of this treatment during the 4 - 6 week period after transplanting. The application of FF and PB at head initiation had no effect on growth rate when measured by dry weight accumulation (Figure 1 D).

Table 1. *Morphological attributes and final plant part dry weights (mean ± SEM) of mature broccoli plants from Experiment 1. Foliar boron was applied (+B) or withheld (-B) to main plots split into three growth rate treatments, HG (19 512 plants ha^{-1} + 198 kg N ha^{-1}), CG1 (32 520 plants ha^{-1} +198 kg N ha^{-1}) and LG (69 686 plants ha^{-1} + 103 kg N ha^{-1}).*

Treatment	Plant height (mm)	Widest stem width (mm)	Stem / petiole dry weight (g)	Branch dry weight (g)	Lamina dry weight (g)	Head dry weight (g)	Plant dry weight (g)	Plant moisture content (%)
- Boron	421±18	48.1±1.2	93.3±8.0	34.5±6.5	63.9±8.2	62.5±5.6	254.1±27.1	90.0±0.5
+ Boron	415±18	47.7±1.3	84.2±7.9	37.3±9.5	56.8±7.7	60.8±5.7	239.2±27.6	91.2±0.3
LG	485±8b	43.7±0.4a	63.2±2.8a	12.1±2.4a	34.2±1.6a	44.7±3.1a	154.3±6.0a	90.0±0.9
CG1	395±6a	48.5±0.8b	95.6±4.8b	42.9±5.8b	65.3±4.3b	65.5±2.4b	269.3±11.8b	90.4±0.2
HG	373±8a	51.4±1.0c	109.4±5.7c	53.5±7.7b	83.0±4.4c	75.5±5.0b	321.4±13.2c	91.2±0.3
Boron	ns	ns	ns	ns	ns	ns	ns	ns
Density	***	***	***	**	***	***	***	ns
Boron x density	ns	ns	ns	ns	ns	ns	ns	ns

*Significance of the main effects are indicated by: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001. Different letters within each section represent a statistical difference (LSD; *P = 0.05).*

Table 2.*Morphological attributes and plant part dry weights (mean ± SEM) of mature broccoli plants from Experiment 2. Plants were grown at CG2 (32 520 plants ha^{-1}) and VLG (100 000 plants ha^{-1}) densities in a factorial combination with 4 treatments, untreated (UT), complete fertiliser (FF), paclobutrazol (PB) and shade (SH).*

	Plant height (mm)	Widest stem width (mm)	Stem dry weight (g)	Branch dry weight (g)	Head dry weight (g)	Plant moisture content (%)
VLG	520±7b	37.7±0.6b	25.0±1.0b	7.8±1.0b	23.6±1.4b	90.8±±0.3
CG2	457±9a	44.8±0.8a	34.2±2.1a	68.3±6.2a	40.4±2.9a	90.1±0.5
UT	499±20b	40.6±1.9ab	30.8±2.8bc	38.1±15.1	35.3±5.4bc	89.9±0.4a
FF	493±14b	41.5±2.1ab	35.0±3.4c	41.8±11.8	28.7±3.5ab	89.5±0.6a
PB	461±17a	42.9±1.1b	30.0±1.3b	43.0±15.9	39.0±4.5c	90.1±0.4a
SH	506±10b	39.3±1.1a	22.2±1.1a	25.5±8.2	24.4±2.6a	92.1±0.2b
Density	***	***	***	***	***	ns
Treatment	**	*	***	ns	***	***
Density x treatment	ns	ns	ns	ns	ns	ns

*Significance of the main effects is indicated by : *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001. Different letters within each section represent a statistical difference (LSD; *P = 0.05).*

Table 3. *Interaction between leaf and total plant part dry weights (mean ± SEM) with density and growth rate treatments from Experiment 2. Plants were grown at CG2 (32 520 plants ha^{-1}) and VLG (100 000 plants ha^{-1}) growth rates in factorial combination with 4 treatments, untreated (UT), complete fertiliser (FF), paclobutrazol (PB) and shade (SH).*

Density	Treatment	Leaf dry weight (g)	Plant dry weight (g)
VLG	UT	44.2±2.3a	101.2±5.3ab
	FF	49.3±4.3ab	112.2±7.3b
	PB	52.5±3.9ab	116.1±5.2b
	SH	37.9±1.7a	80.2±2.4a
CG2	UT	96.2±3.7cd	263.4±9.1d
	FF	109.1±8.9d	257.1±8.5d
	PB	88.8±10.9c	249.1±13.3d
	SH	62.4±4.5b	164.4±6.1c
Interaction		**	***

*Significance of the main effects is indicated by : *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001. Different letters within each section represent a statistical difference (LSD; *P = 0.05).*

There was no direct relationship between absolute growth rate and either hollow stem incidence or severity in either Experiments 1 or 2. In Experiment 1, absolute growth rate could be used as a predictor of the probability of hollow stem affecting >5% of the stem (Hosmer Lemeshow test $P = 0.1$; see Figure 2) with the probability of hollow stem occurring in an individual plant increasing 5 fold (Exp β) for every 1 gram day^{-1} increase in absolute growth rate. The predictive equation

$$\text{Log}\,(p\,/\,p\text{-}1) = -3.428 + 1.645 * \text{AGR}$$

where p is the probability of hollow stem affecting more than 5% of the stem and AGR is the absolute growth rate, predicted the absence of hollow stem 77% of the

time and its occurrence 90% of the time, with an overall predictive capability of 86%. This indicates that the development of hollow stem was in this instance linked to growth rate. A similar relationship was not present in the data recorded in Experiment 2, even when the PB treated plants were excluded from the data, or when UT plants were considered alone.

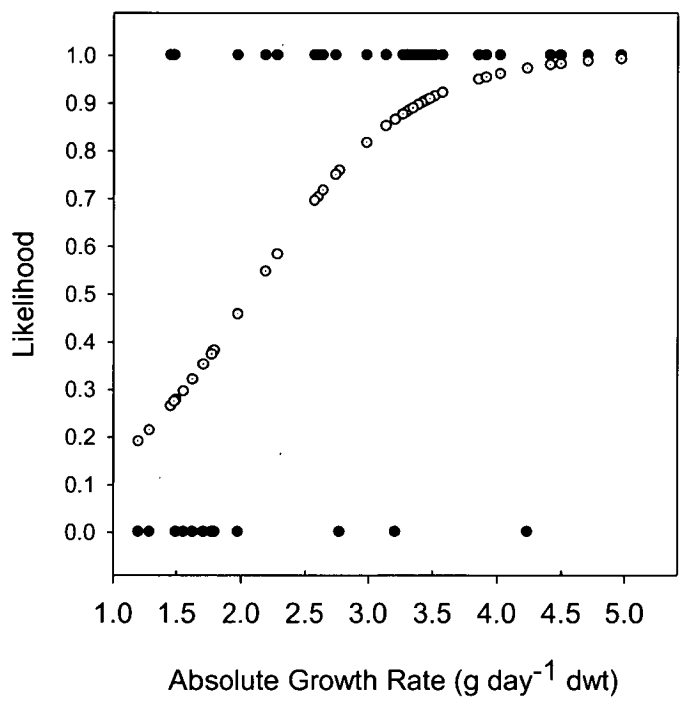


Figure 2. *Logistic binomial regression of the observed incidence of hollow stem affecting greater than >5% of the stem tissue (•) and the predicted likelihood of hollow stem occurring (•) plotted against the absolute growth rate (g day $^{-1}$ dry weight) calculated between transplant and harvest.*

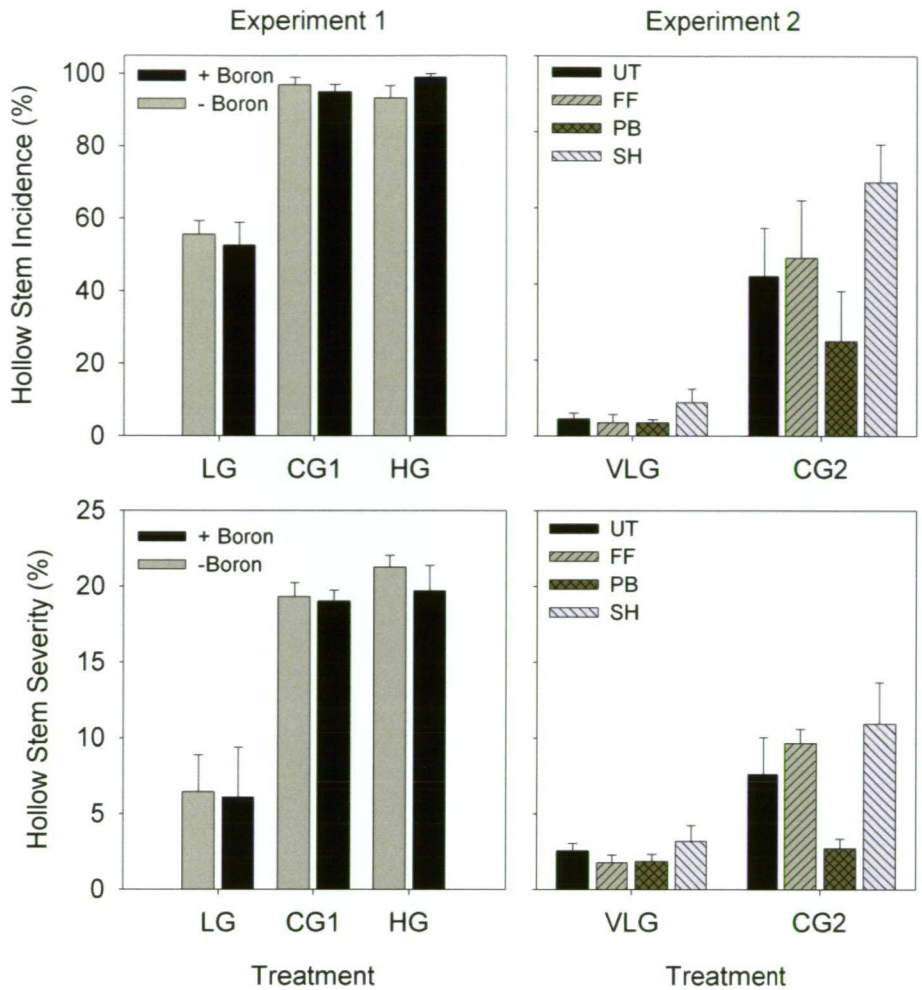


Figure 3. *Hollow stem incidence and severity of mature broccoli plants harvested in experiments 1 & 2. Experiment 1. Foliar boron was applied to (+Boron) or not applied (-Boron) to main plots split into growth rate treatments; HG (19 512 plants ha^{-1} + 198 kg N ha^{-1}), CG1 (32 520 plants ha^{-1} + 198 kg N ha^{-1}) and LG (69 686 plants ha^{-1} + 103 kg N ha^{-1}). Experiment 2. Plants were grown at two growth rates (CG2; 32 520 plants ha^{-1}; VLG; 100 000 plants ha^{-1}) in factorial combination with 4 growth rate treatments, untreated (UT), complete foliar fertiliser (FF), paclobutrazol (PB) and shade (SH). Error bars are SEM.*

SHADING REDUCED GROWTH RATE BUT NOT THE INCIDENCE OR SEVERITY OF HOLLOW STEM

In Experiment 2, both the AGR and consequently final plant dry weight were suppressed by the application of SH at inflorescence initiation but not by the other treatments (Table 2, Figure 1). SH reduced dry weight accumulation at the CG2 rate but not at the VLG rate when compared to the UT plants. AGR was however significantly reduced at both the CG2 and VLG rates from weeks 6 to 10, with no

interaction present. Despite the significant reduction in AGR and final plant dry weight under SH applied during primordial head development, the incidence or severity of hollow stem was not reduced when compared to the UT plants.

PACLOBUTRAZOL REDUCED HOLLOW STEM INCIDENCE AND SEVERITY

The foliar application of PB did not reduce total dry weight accumulation (Table 3) or AGR (Figure 1) when compared to UT plants at either level of growth rate. Even though growth rate was not suppressed, the severity of hollow stem at the CG2 rate was reduced by PB to levels comparable to that of all treatments at the VLG rate (Figure 3). The reduction in hollow stem incidence in response to PB was not as dramatic and was only significantly ($P = 0.05$) less than the SH treatment.

PLANT MORPHOLOGY AND PARTITIONING

Growth rate influenced plant morphology and dry matter accumulation. Low growth treatments (highest planting densities & shading) led to plants with longer, thinner stems with reduced dry weights (Table's 1, 2 & 3). CG1, CG2 and HG rates increased leaf and inflorescence dry weights and had a large influence on the partitioning of plant dry matter devoted to axillary branch production. The application of boron as Solubor (+ Boron) or as FF did not have an influence on plant morphology or dry matter partitioning. SH plants were the same height as UT plants but had reduced stem and inflorescence dry weights and marginally higher plant moisture content. This treatment also resulted in lower leaf dry weight under the CG2 regime but no significant ($P = 0.05$) effect from shading was observed under VLG. Plants treated with PB were shorter than UT and SH plants.

The growth rate treatments also influenced the partitioning of plant dry matter in both experiments. Both the HG and CG1, CG2 treatment plants diverted a significantly greater proportion of dry weight to the production of axillary branches (Table's 4 & 5). The increased diversion of dry matter to axillary branch production when comparing the LG to CG1 and VLG to CG2 was 8.0% and 21.8% of total dry matter respectively. This diversion was at the expense of dry matter

allocation to the stems, leaves (Experiment 1 only) and inflorescence. When considering the dry matter diverted to axillary branch production at CG1 and CG2 as a whole, 100% and 70% respectively was directed away from the stems and heads as opposed to diversion from leaf production. While the treatments affected dry matter allocation, there was no direct or probabilistic relationship between the incidence and severity of stem cavitation and branch dry matter or partitioning (Data not shown). The application of FF, PB or SH did not in general influence dry matter partitioning, with the exception of head dry matter allocation which was reduced by FF.

Table 4. *Partitioning of plant dry matter (mean ±SEM) from Experiment 1. Foliar boron was applied (+B) or withheld (-B) to main plots split into three growth rate treatments, HG (19 512 plants ha-1 + 198 kg N ha^{-1}), CG1 (32 520 plants ha-1 + 198 kg N ha^{-1}) and LG (69 686 plants ha^{-1} + 103 kg N ha^{-1}).*

Treatment	Stem partition (%)	Branch partition (%)	Leaf partition (%)	Head partition (%)
- Boron	37.52±1.25	12.71±1.34	24.61±1.01	25.16±1.02
+ Boron	36.36±1.82	13.91±2.79	23.55±1.08	26.18±1.32
LG	41.09±1.46a	7.86±1.61a	22.17±0.65	28.88±1.21a
CG1	35.51±1.01b	15.84±1.78b	24.27±1.13	24.38±0.61b
HG	34.08±1.37b	16.56±2.28b	25.92±1.45	23.44±0.92b
Boron	ns	ns	ns	ns
Density	**	*	ns	*
Boron x density	ns	ns	ns	ns

*Significance of the main effects is indicated by : *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001. Different letters within each section represent a statistical difference (LSD; *P = 0.05).*

Table 5. *Partitioning of plant dry matter (mean ±SEM) from Experiment 2. Plants were grown at CG2 (32 520 plants ha^{-1}) and VLG (100 000 plants ha^{-1}) growth rates in factorial combination with 4 treatments, untreated (UT), complete fertiliser (FF), paclobutrazol (PB) and shade (SH).*

Treatment	Stem partition (%)	Branch partition (%)	Leaf partition (%)	Head partition (%)
VLG	24.62±0.47b	7.43±0.90b	44.91±0.72b	23.04±0.79b
CG2	14.92±0.51a	29.20±2.00a	38.34±1.47a	17.55±0.93a
UT	20.66±2.11	16.84±4.66	40.78±1.66	21.71±1.13b
FF	20.87±2.05	19.55±3.86	42.96±1.38	16.62±1.39a
GS	18.35±1.95	18.44±5.80	40.36±2.61	22.86±1.84b
SH	19.91±1.73	16.88±4.29	42.72±2.19	20.50±0.82b
Density	***	***	***	***
Treatment	ns	ns	ns	***
Density x treatment	ns	ns	ns	ns

*Significance of the main effects is indicated by : *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001. Different letters within each section represent a statistical difference (LSD; *P = 0.05).*

DISCUSSION

The incidence and severity of hollow stem was higher at high plant growth rates, which were mediated by plant stand density. This is consistent with previous studies that have demonstrated a positive correlation between hollow stem incidence and plant growth rate for cauliflower (Everaarts and Putter 2003) and broccoli (Zink and Akana 1951; Gorski and Armstrong 1985). In these studies different rates of growth were also induced by varying plant stand density. In our first trial the probability of hollow stem affecting more than 5% of the stem could be predicted by absolute growth rate expressed as total plant dry weight accumulation (g day^{-1} dwt). In the second study, assessment of a greater range of treatments to manipulate plant growth rate independent of plant stand density revealed no direct link between plant growth rate and hollow stem incidence and severity. In addition, while growth rates at the commercial planting densities were

similar between trials, the incidence and severity of hollow stem was greater in the first season.

The inconsistency in the relationship between growth rate and hollow stem has been noted by other workers (Tremblay 1989). In this study, hollow stem severity was significantly greater in the year where growth rates were lowest and maturity had been delayed by 15 days via a seasonal effect. In a subsequent study in broccoli, hollow stem incidence increased with N application rates made at 5 weeks in one season yet in the following season, despite a yield response to N application at 5 weeks, and higher total yields than the previous year, hollow stem incidence was minimal for all N application treatments (Coulombe et al. 1999). This indicates that a factor, or factors, other than absolute growth rate are responsible for the development of hollow stem.

The hypothesis that an alternative mechanism may be involved is also supported by the suppression of hollow stem severity at the commercial growth rate by paclobutrazol, despite this compound not having influenced growth rate when measured as dry weight accumulation (although it should be noted that plant height was reduced). In a study conducted on beans (*Phaseolus vulgaris* L.) (Takano et al. 1995), hollowing of the first internodes was similarly reduced by uniconazole, another related gibberellin biosynthesis inhibitor in the triazole group. Also in support of this assertion is that while shading the plants from head initiation onwards did reduce growth rate, this considerable reduction did not reduce the incidence or severity of cavity formation.

The role of boron in the development of hollow stem has been controversial for many years, this debate being noted from as early as 1940 by Chandler. The application of boron as Solubor in the first trial and as part of a complete FF in the second trial did not influence the severity nor incidence of hollow stem. This data, although not conclusive, lends support to other field studies which have reported no direct influence of boron on the development of stem cavities in broccoli (Gupta and Cutcliffe 1973; Hipp 1973; Gupta and Cutcliffe 1975; Vigier and Cutcliffe 1984; Tremblay 1989; Scaife and Wurr 1990; Griffith and Carling 1991). While there is no doubt the stem cavities in broccoli do form in response to severe boron

deficiencies induced under glasshouse conditions, these cavities are always associated with a complex of other symptoms. The majority of field studies concerned with hollow stem do not report these additional symptoms, nor with the exception of the browning of stem tissue, were they observed in this study. While boron deficiency may have a role in hollow stem development, clearly other mechanisms affect the incidence and severity of the disorder.

While absolute growth rate is not a causal factor, processes that are associated with the rate and pattern of growth may be involved in the initiation of hollow stem. The growth rate treatments mediated by planting density influenced the morphology of the plant and the partitioning of dry matter. In particular, a much greater portion of dry matter was allocated toward axillary branch production at the highest growth rates. This diversion of plant resources to axillary branching was at the expense of leaf, stem and inflorescence dry matter accumulation. Carr and Jaffe (1995) proposed that the development of stem cavities in herbaceous dicotyledons such as beans (*Phaseolus vulgaris*), tomato (*Lycopersicon esculentum*), and buckwheat (*Fagopyrum esculentum*) is driven by a shortfall in photosynthate supply to developing sinks. They provided some evidence to support the hypothesis that, in some species at least, where photosynthesis cannot meet the demands of a developing sink, carbohydrates stored in the pith are recycled via autolysis of the stem pith cells. Pith cell autolysis in response to demands from the developing inflorescence may provide an alternative explanation to both the boron and growth rate hypotheses.

These results add to the weight of literature which suggests that a boron deficiency is not the primary mechanism leading to the development of stem cavities in field grown broccoli and that the development of this disorder is indirectly related to growth rate.

CHAPTER 9

DIFFERENTIAL STEM TISSUE GROWTH: A FUNDAMENTAL MECHANISM

INTRODUCTION

Hollow stem in broccoli can be induced by agronomic treatments that promote high plant growth rates, such as low plant stand density or high nitrogen application rates (Chapter 8) (Zink and Akana, 1951, Griffith and Carling, 1991, Cutcliffe, 1972). Yet the effects of growth rate on hollow stem incidence and severity have not always been consistent (Chapter 8, Tremblay, 1989, Coulombe et al., 1999) suggesting that other underlying factors mediate this response. Two alternative hypotheses have been proposed; the first, that cavity development in plants might be related to pith autolysis driven by carbohydrate reallocation (Carr and Jaffe, 1995) and, secondly, that mechanical tissue stress within the stems of plants with higher growth rates, might lead to the development of stem fractures (Griffith and Carling, 1991).

Under the pith autolysis hypothesis, it is proposed that cellular degradation occurs in response to an increased demand for assimilates (Carr and Jaffe, 1995). In the case of broccoli, hollow stem may arise from the reallocation of starch stored in the pith cells to support the rapid growth of the developing inflorescence. The initial appearance of watery tissue in the central pith, and in severe cavities, the apparent disappearance of pith tissue and the sculptured appearance of the walls, (Chapter 7) provides anecdotal evidence that pith autolysis might be involved in cavity development. This hypothesis is to some extent supported by the observed action of paclobutrazol (Chapter 8). Gibberellin has been implicated as a promoter of programmed death of aleurone cells in barley (Bethke et al., 1999). Although the role of GA in this context is possibly specific to seed germination, the effect of paclobutrazol (Chapter 8), a gibberellin biosynthesis inhibitor, suggests that a similar mechanism could be responsible for cavity development via programmed

cell death of the starch storing parenchyma cells within the pith. However, there is only limited quantitative evidence (Chapter 7) that the reallocation of starch from within the stem pith tissue is associated with the development of hollow stem, and it seems unlikely that this process alone could explain the cell fracture and separation observed to occur during this process.

An alternative hypothesis is that mechanical strain developed within the stem during inflorescence development can be sufficient to induce stem cracking (Griffith and Carling, 1991). The distinct layers of tissue within plant stems and their different mechanical properties have been shown to contribute to longitudinal tensile strain and compression of the inner pith tissues (Hejnowicz, 1997, Brown et al., 1995). For the sunflower hypocotyl, the epidermal tissue has been shown to undergo both longitudinal tensile stress and transverse tangential stress. In contrast, the inner pith tissue experienced compressive stresses that were longitudinal, tangential and radial in orientation (Hejnowicz, 1997). Consequently, if a similar pattern occurred in brassica flowering stems, the development of differential strain along these axes of the anisotropic stem tissues of broccoli (and cauliflower), may lead to the failure of some of these tissues, as has been observed in other horticultural species. The fracture of the phloem parenchyma in carrot tissue has previously been attributed to growth stress and gradients in turgor, and the fact that only this tissue and not the xylem fractures, attests to the role of the different mechanical properties of these tissues (Gracie and Brown, 2004). Although the anatomical location of the various tissues in broccoli is different, the fundamental forces at play are likely similar. Plant growth regulators such as paclobutrazol act by reducing cell elongation and lowering the rate of cell division (Rademacher, 2000), and in the case of uniconazole, this has been linked to an increase in the cell wall yielding coefficient and yield threshold (Davis and Curry, 1991). The resultant reduction in inter-nodal elongation, and other possible effects of paclobutrazol might explain its role in reducing the severity of stem cavities in broccoli if this latter process is underpinned by growth induced mechanical stress within the stem.

The previous description of hollow stem aetiology also provided some qualitative evidence that suggests mechanical stress related to plant development may be

involved in the initiation of stem cavities (Chapter 7). Hollow stem begins just prior to buttoning as a fine transverse fracture at the top of the stem, in close proximity to the inflorescence. This fine split either bisects the central pith cylinder producing a linear fissure, or propagates along its circumference producing a crescent shape fracture. The fine split observed during the early stages of hollow stem, and the subsequent longitudinal propagation through the pith tissue as cavity development progresses gives the appearance of tissue failure generated by mechanical stress. Additionally, the later appearance of elliptical secondary cavities in the wall of the primary edifice might be generated by longitudinal stresses within the stem tissue. The cell separation and fracturing observed in the fractured tissues was also indicative of mechanical stress and strain.

The hypothesis explored in this study was that differential mechanical stresses generated across the anisotropic broccoli stem caused pith tissue to fracture in a pattern that was consistent with the initial stages of hollow stem.

METHODOLOGY

Plants used in this study were sampled between buttoning stage of development (10mm inflorescence) and commercial harvest maturity from two commercial crops at West Pine, Tasmania. In both crops, plants of 'Marathon' were grown from transplants in two rows 400 mm apart on 1.64 m beds at a density approximating 34 000 plants.ha^{-1}. Plants were removed from the soil with the root ball intact, the leaves removed with a sharp blade and the stem then placed in a sealed plastic bag. All plants were randomly sampled from a 150 m^2 area within each crop that was representative of the entire crop. Plants were then transported to the laboratory and kept at 3 ^{0}C until being processed on the same day.

TISSUE SECTIONING AND INCUBATION

Tissue sections were taken from five regions along the length of the stem; just above node 5 (lower), half way along the length of the stem (middle), the midpoint between the middle and upper stem (upper middle), the region just below the lowest inflorescence branch (upper) and from between the lowest inflorescence branch and the point where the higher positioned II order branches diverged into single entities (head). The stem pith tissue in a transverse section of stem was typically composed of a region of white tissue (central pith) which was then encircled by chlorophyll containing pith tissue that extended to the vascular and cortex tissue located at the perimeter of the stem.

The pith tissue between the central pith and the vascular tissue was viewed as two equal halves. The region immediately adjacent to the central pith was referred to as inner pith, while the tissue adjacent to the vascular tissue was termed outer pith. The stem tissue sections were subject to various treatments depending on the experiment and incubated for 24hrs in a plastic petri dish containing deionised water. Care was taken to ensure the specimens were not entirely submerged with water levels generally at 80% of the specimen's height.

DIGITAL PHOTOGRAPHY

Data relating to cavity formation and tissue extensibility before and after incubation was captured using digital image analysis. Photographs were taken of each specimen from a consistent height using a Power Shot G5 (Canon Inc.) compact digital camera mounted on a stand. A scale bar was included in each shot and the zoom was set to the same focal length (e.g. 25.09 mm) for each session within a trial. There were small variations in the focal length between trials and consequently the resolution of each pixel varied from 17 to 22 pixels per mm across the various trials. Images were collected prior to each treatment, and at each stage of assessment. Image analysis was conducted using Fovea Pro 4.0 (Reindeer Graphics), a plug in to Adobe Photoshop CS2 (Adobe Systems Inc.).

DE NOVO GENERATION OF STEM FRACTURES

Transverse discs *ca.* 4.5 mm thick were sectioned from the lower, middle, upper middle and upper regions of 5 plants randomly sampled on the 11th June 2008. The untreated sections were placed in deionised water and incubated for 24 hrs. For each section the existence of a stem cavity prior to and after incubation was recorded in addition to the length (mm), width (mm) and area (mm^2) of each fissure.

CIRCUMFERENTIAL TENSION

Circumferential tension of various stem tissues was estimated using sub sections of a 40 mm region from the lower, middle and upper sections of 5 stems collected on the 23rd April 2008. The release of circumferential tension was measured for an entire transverse slice, the central pith cylinder, inner pith and outer pith combined and the vascular / cortex regions (Figure 1). Tension stored in an entire transverse section 4.5 mm thick from each stem section was estimated by making a radial incision from the centre of the disc through to its perimeter (Figure 1A). The immediate release and release after 24 hrs incubation of circumferential tension was then measured using a gap index (Sorensen and Harker, 2000) calculated as

$$I_g = \frac{P - P_0}{P_0}$$

where P_0 is the original perimeter, and P is the final perimeter after treatment. The central pith, inner and outer pith combined and vascular / cortex tissues were excised from a second transverse disc of tissue and the gap index measured after a radial incision through the respective tissues (e.g. Figure 1B).

TISSUE EXTENSION AND EXTENSIBILITY

Tissue extension was measured across the pith tissues taken from *ca.* 4.5 mm thick transverse discs of the lower, middle and upper sections of 5 stems collected on 29th June 2008. Sections of tissue 10 mm long (tangentially oriented) and 4.5 mm

wide (radially oriented) were then sectioned from the central pith, inner pith, outer pith and vascular / cortex regions (Figure 1C). The radial and tangential faces were marked (Sharpie Fine Point Permanent Marker, Sanford Inc.) to ensure the same surface was oriented towards the camera for each image capture. Measurements of the length (mm) of the tangential, radial and longitudinal faces of

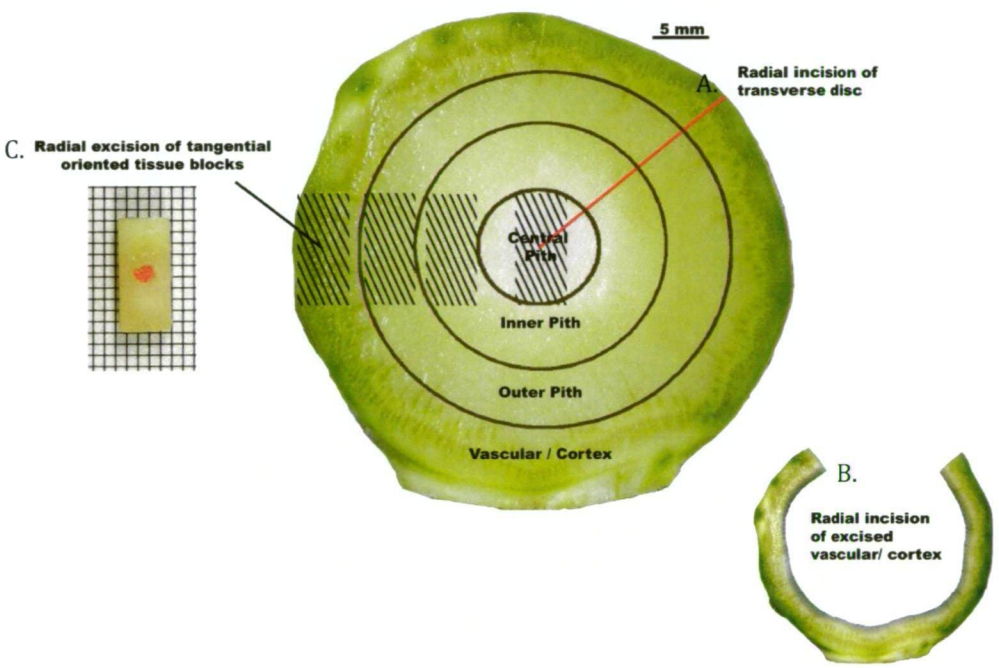


Figure 1. *Diagram illustrating the different treatments applied to transverse sections of broccoli stem. Treatments included* ***(A)*** *radial incision* ***(B)*** *excision of the vascular cortex, central inner and outer pith as rings of tissue, and* ***(C)*** *radial excision of tangentially oriented sections from the same area.*

each tissue block were made at the time of excision and after incubation. Relative tissue extension was calculated as:

$$E_{H_2O} = \frac{D_{24} - D_0}{D_0}$$

where D_0 is the relevant dimension (tangential, radial or longitudinal) at the time of incision and D_{24} the resultant dimension after 24 hours incubation.

Tensile strength and strain (ε) measurements were also made of the central pith tissue using an Instron Materials Testing Machine (Instron Inc). The procedure was based on that previously used for carrot parenchyma tissue (McGarry, 1995). Plants were sampled on the 13th June 2008, placed on ice and transported overnight to the laboratory. Strips of tissue 5 mm (tangential) x 2 mm (longitudinal) were excised from transverse discs of the lower, middle and upper sections of 9 stems. Each specimen was then clamped at a length of 10 mm (radial) and extended at a rate of 1.67×10^{-5} m s^{-1}. During initial testing the tissues repeatedly failed in areas other than the central pith. Subsequently, to ensure failure in this central region, edge notches were cut to a depth of 1 mm in the centre of the strip (McGarry, 1995). Results were reported as load (N) once per second.

Tensile strength (breaking strength) was determined as the maximum stress (MPa) achieved at failure. Stress was calculated as;

$$\sigma = \frac{T}{A}$$

where T is the load (N) and A is the cross sectional area (mm^2).

Extensibility was defined as the maximum strain at which failure occurred (breaking strain). Strain, the ratio of change in length to original length was calculated as;

$$\varepsilon = \frac{L - L_0}{L_0}$$

where L is the final length, L_0 the length at time 0.

DIURNAL EXTENSIBILITY

Diurnal fluctuations in stem diameter were monitored on three plants using 4 linear variable differential transformer sensors (LVDT; Solarton model SM3, ± 3 mm stroke range; RS components Ltd, London) from 7-14th June 2008. Each sensor was attached to a

single channel LVDT transducer conditioner (Solarton, model OD5) and the output recorded on a voltage channel of a LI-COR data logger (model LI-1400, LI-COR, Lincoln, NE). The plants monitored were located within a 3.28 m x 5 m plot. A digital micrometer accurate to three decimal places was used to create a calibration curve between displacement and the voltage output for each sensor.

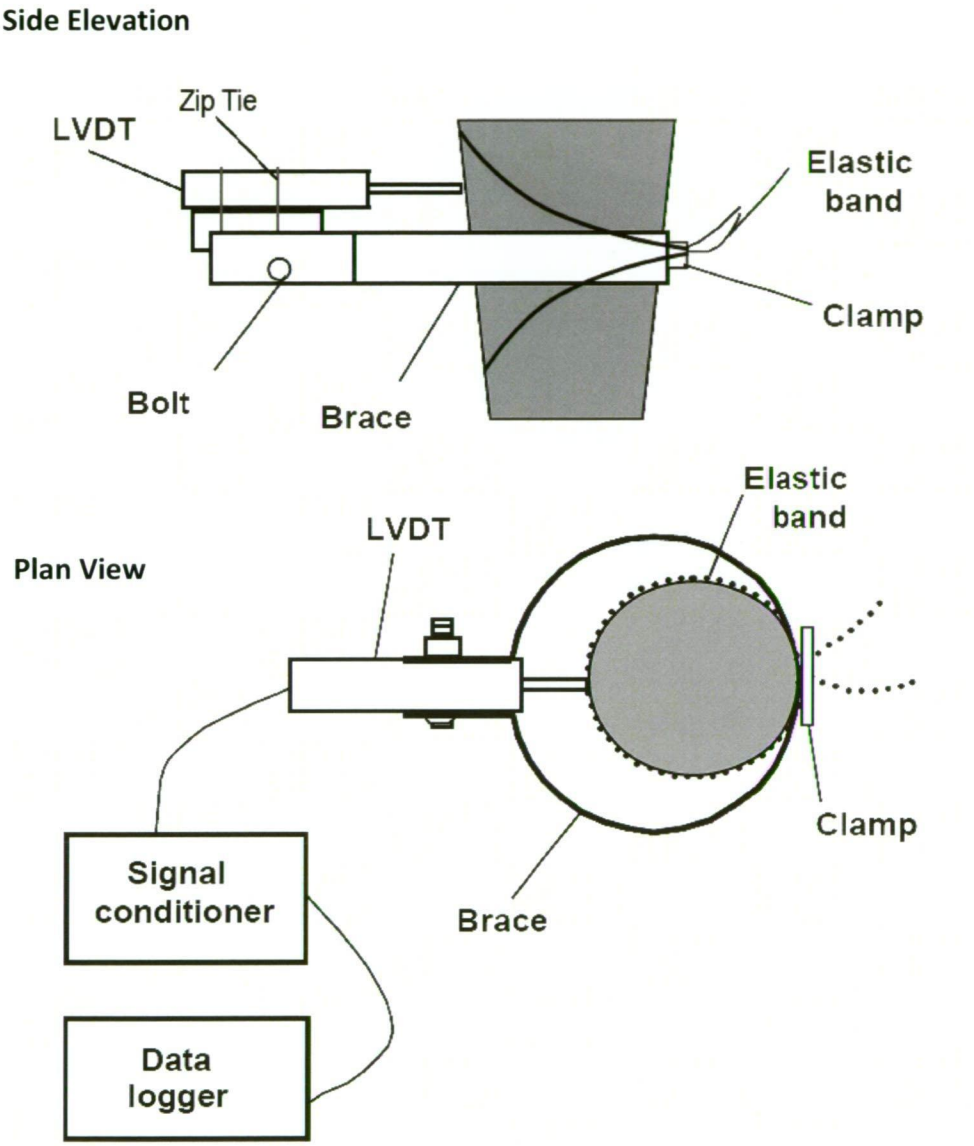


Figure 2. *Diagram of LVDT sensor mounted against the upper middle stem section (modified from Gracie and Brown, 2004). The use of the ties allowed for small adjustments in the position of the casing while being sufficiently strong to hold the sensor still during operation. The sensor mount was held away from the proximal surface of the stem by a 60 mm pipe clamp which was itself held against the distal surface by an elastic tie looped around the stem above the sensor. A second elastic tie was looped around the stem below the bracket to stop vertical movement of the assembly.*

This curve is sigmoid in nature and all measurements were made within the linear section of the curve, from -1.7 V to +1.7 V. Small modifications were made to a previously described system (Gracie and Brown, 2004) to mount the sensor on the broccoli stem (Figure 2).

The LVDT system was assembled so as to allow movement of the sensor mount with the stem while holding the casing at a set distance from the stem. The armature of the sensor was spring loaded to hold it against the stem. This configuration allowed the sensor mount to move with the stem under windy conditions while the relative position of the armature remained unchanged unless affected by fluctuations in stem diameter. Additionally, this design did not restrict expansion of the stem. Placement of the bracket (pipe clamp) required the removal of a leaf directly below, but at a reasonable distance from the region in which the sensor was mounted.

Sensors 1& 2 were used to measure the flux in diameter at the 'upper middle' and 'upper' stem positions of the same plant. The stem widths at these locations were 100% and 87% of the widest point of the stem at the end of the trial period. Sensors 3 & 4 were positioned in the 'upper stem', the widths of these two locations being 83% and 100% of the widest point of the stem.

On the morning of 13th June 2008, 5 plants were randomly sampled from within the plot at 0600 hrs. Plants were defoliated and placed in bags as described earlier and transported to the laboratory. Transverse discs of tissue were then taken from the lower, middle, upper middle and top sections of the stem and assessed for the immediate release of circumferential tension. A second transverse disc of tissue was removed from each stem section and assessed for extensibility of the central pith, inner pith, outer pith, vascular / cortex and epidermal tissues using the water incubation technique. The same procedure was employed for a further 5 stems sampled from within the plot at 1300 hrs on the same day. Vapour pressure deficit (VPD) was calculated from temperature and humidity measurements recorded at 15 minute intervals at the Forthside Vegetable Research and Demonstration farm at Forth, Tasmania. VPD (kPa) was calculated as;

$$VPD = e_{sat} - e_{air}$$

where e_{sat} is the saturation vapour pressure and e_{air} the air vapour pressure. The saturation vapour pressure (e_{sat}) was approximated (Murray, 1967) using;

$$e_{sat} = 0.61078 \exp\left[\frac{17.269T}{237.3+T}\right]$$

where T is the air temperature in degrees Celsius.

STATISTICAL ANALYSIS

Analysis of variance was used to compare means using the Two Way ANOVA routine of Genstat Ver. 10. Range testing was conducted using the least significant difference test. Where the data did not meet the assumptions of this model, the means were compared using the non parametric K independent samples procedure in SPSS Ver 16.0 with Kruskal Wallis H selected as the test type.

RESULTS

DE NOVO SYNTHESIS OF STEM FRACTURE

Hydration of intact transverse slices of broccoli stem resulted in the formation of de novo fractures in the central pith tissues. Three of the five stems sampled had existing cavities in different configurations through the upper middle, upper and head pith tissues (Table 1). After incubation, the remaining two stems also developed fractures in the transverse slices of the same region (Figure 3) and in those stems with cavities, new fractures developed in the stem sections previously unaffected. No cavities were observed in the middle or lower stem pith tissues before or after treatment in any of the stems. Hydration of the pith tissues increased the cross sectional area of all the pre-existing cavities, due mainly to an increase in the length of the fissure (Table 2).

Table 1. *The presence (X) or absence (-) of pre-existing and de novo stem cavities in 5 broccoli stems prior to hydration and after 24 hrs. De novo cavities are highlighted in grey.*

	Stem 1		**Stem 2**		**Stem 3**		**Stem 4**		**Stem 5**	
Section	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr
Lower	-	-	-	-	-	-	-	-	-	-
Middle	-	-	-	-	-	-	-	-	-	-
Upper	X	X	-	X	X	X	-	X	-	X
Upper Middle										
Upper	-	X	X	X	-	-	-	X	-	X
Head	X	X	-	-	-	X	-	X	-	-

Table 2. *Effect of hydration (24 hours) on existing cavity size and the dimensions (mean ± SEM) of de novo cavities. The dimensions reported for existing fractures are the increase in size after incubation while those for de novo fractures are the final dimensions.*

Stem Section	**Existing fracture number and *increase* in dimensions**				**De novo fracture number and final dimensions**			
	n	Length (mm)	Width (mm)	Area (mm)	*n*	Length (mm)	Width (mm)	Area (mm)
Lower	0		0	0	0	0	0	0
Middle	0		0	0	0	0	0	0
Upper- Middle	2	3.76 ±1.09	0.44 ±0.21	7.76 ±4.82	3	8.68 ±2.30	0.64 ±0.21	7.29 ±3.90
Upper	1	4.30	0.97	12.09	3	12.87 ±2.39	0.91 ±0.15	15.24 ±4.42
Head	1	5.11	0.80	12.10	2	9.31 ±0.36	0.78 ±0.18	8.17 ±3.50

The cavities developed de novo were initiated in the central pith tissue and were either linear, passing through the centre of the region, on crescent shaped in nature, with the curve linear axis of the fissure following the boundary of this regions tissue. This observation also held true for pre-existing fractures. None of the pre-existing or de novo fractures extended beyond the central pith into the chlorophyll containing inner / outer pith tissues, either before or after hydration. The largest de novo fractures occurred in the upper stem ($P \leq 0.01$).

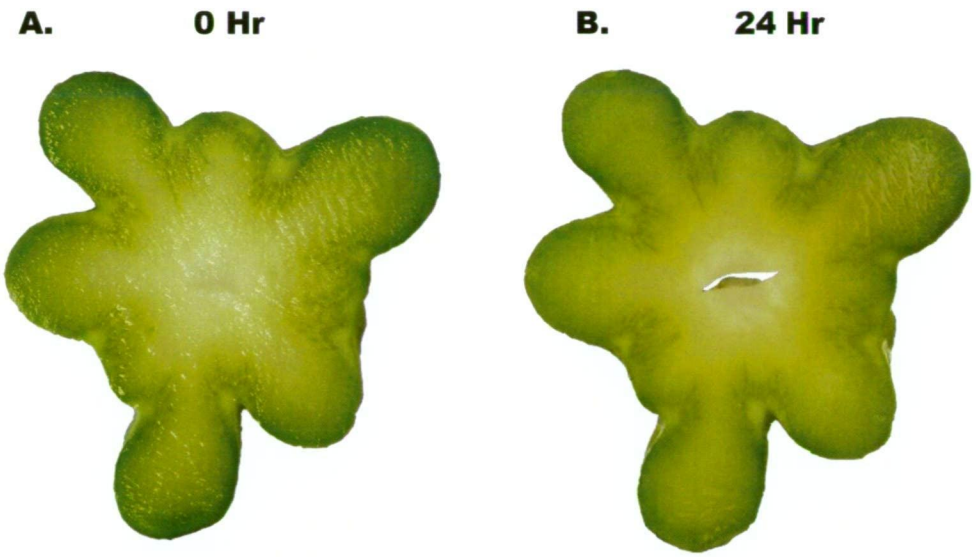


Figure 3. *De novo synthesis of a stem fracture in the inflorescence pith tissue (head) of Stem 4* ***(A)*** *before incubation and* ***(B)*** *after 24 hours incubation in deionised water.*

CIRCUMFERENTIAL TENSION STUDY

There were significant differences in the release of circumferential tension prior to, and after hydration between the different stem sections ($P \leq 0.001$), tissue types ($P \leq 0.001$) and an interaction between these two factors ($P \leq 0.001$). Circumferential tension was highest in the vascular cambium / cortex tissue of the outer stem, with a significant release of strain energy occurring immediately after incision (Table 3). An even greater release of strain energy was recorded in these tissues after 24 hr incubation. The middle and upper stem sections exhibited the highest levels of strain release, this being much lower in the vascular / cortex tissues of the lower stem, particularly after hydration. A small amount of strain was released in the transverse slice after incubation and only insubstantial strain release occurred in the central and inner / outer pith tissues.

Table 3. *The release in circumferential tension reported as the gap index (mean ± SEM) immediately after incision of the tissues and after 24 hours hydration in deionised water.*

Tissue Type & Location	Gap Index	
	0 hr	24 hr
Transverse Slice		
Lower	0.01 ± 0.00	0.06 ± 0.01
Middle	0.01 ± 0.00	0.06 ± 0.02
Upper	0.00 ± 0.00	0.01 ± 0.00
Central Pith		
Lower	0.00 ± 0.00	0.01 ± 0.00
Middle	0.00 ± 0.00	0.00 ± 0.00
Upper	0.00 ± 0.00	0.00 ± 0.00
Inner + Outer Pith		
Lower	0.00 ± 0.00	0.03 ± 0.02
Middle	0.00 ± 0.00	0.00 ± 0.00
Upper	0.00 ± 0.00	0.00 ± 0.00
Vascular + Cortex		
Lower	0.08 ± 0.02	0.19 ± 0.05
Middle	0.16 ± 0.02	0.56 ± 0.05
Upper	0.13 ± 0.02	0.61 ± 0.05
Max LSD ($P = 0.05$)	0.035	0.092

In the diurnal trial, the immediate release of circumferential tension in the vascular / cortex tissues was influenced by stem position ($P \leq 0.001$) but not by the time of day ($P = 0.844$). The tension stored by these tissues increased from relatively low levels to peak in the upper middle and upper stem regions (Figure 4) and was approximately twice as great as that observed earlier (Table 3).

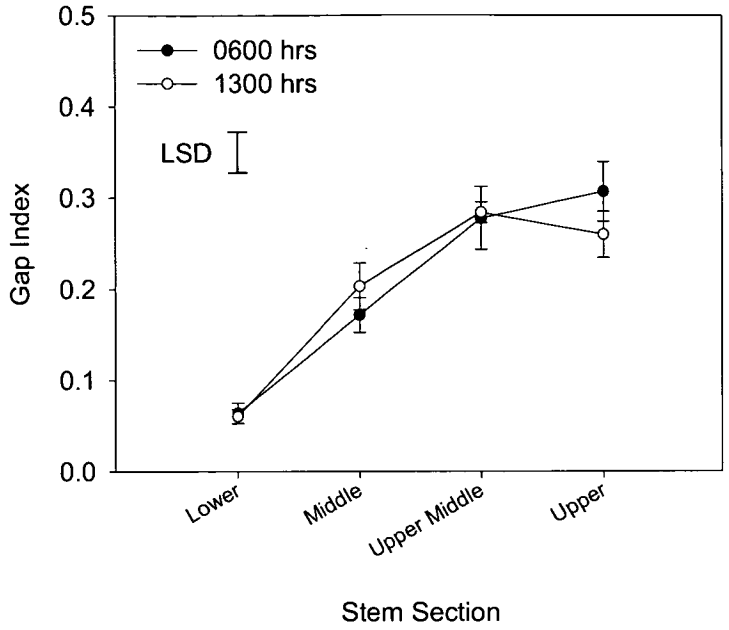


Figure 4. *Release of circumferential tension presented in the vascular / cortex tissues at 0600 hrs and 1300 hrs by stem position. Data points are the Gap Index (mean ± SEM;* n = *5).*

TISSUE EXTENSIBILITY

Radial, tangential and longitudinal tissue extension was affected by the tissue type and its position within the broccoli stem (Figure 5). There was a significant interaction ($P \leq 0.001$) in radial extension (Figure 5A) between stem position ($P \leq 0.05$) and tissue types ($P \leq 0.05$). Radial extension within the lower stem was greatest in the central and inner pith tissues, but then declined through the outer pith tissue to having only limited extensibility in the vascular / cortex tissues. In contrast, in the middle and upper stems radial extension was almost negligible in the central and inner pith regions and in some cases tissue contraction was evident. Radial extension in the middle and upper stem then increased through the outer pith to peak in the vascular cortex tissue.

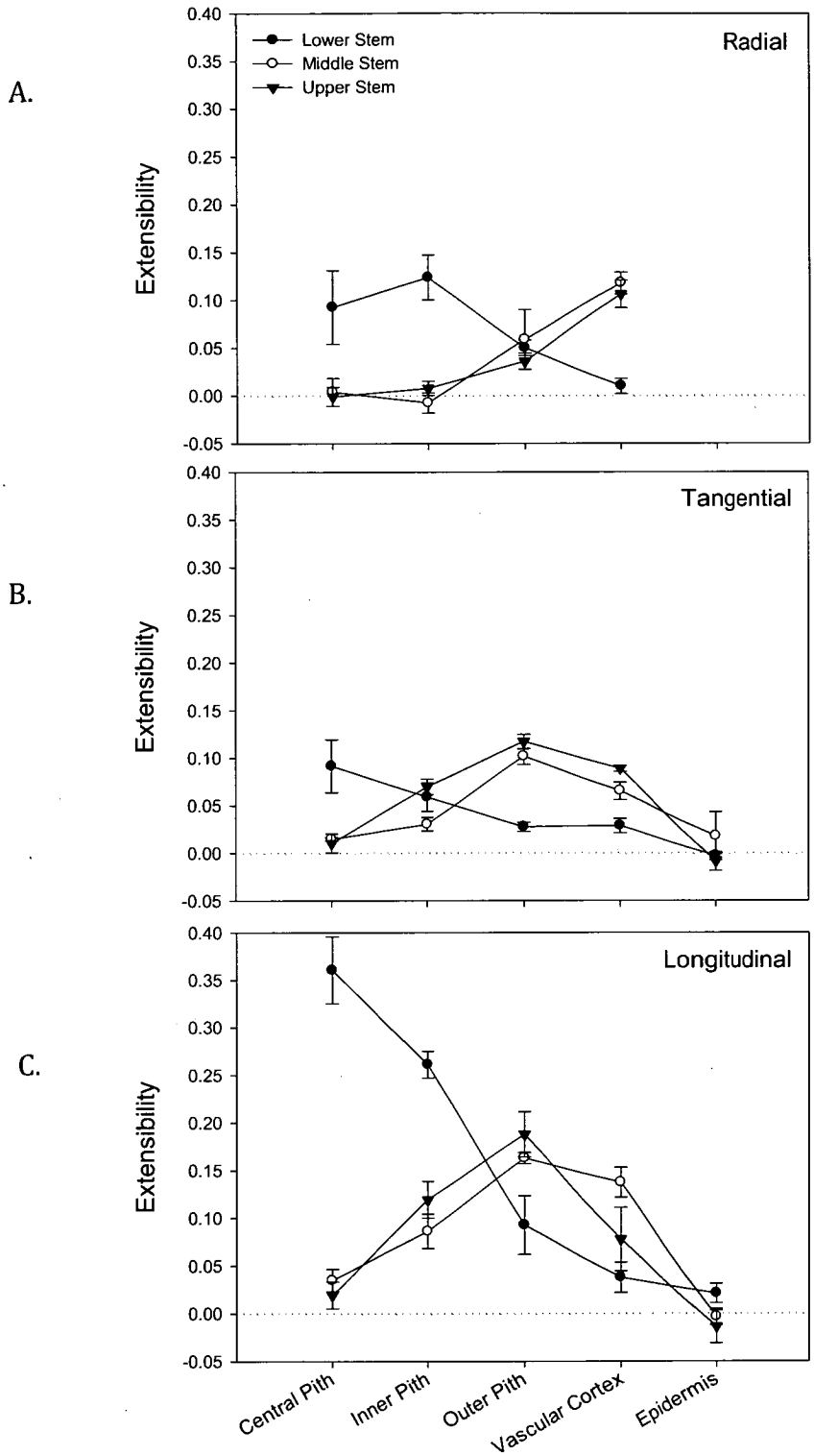


Figure 5. *Mean (± SEM)* ***(A)*** *radial,* ***(B)*** *tangential and* ***(C)*** *longitudinal relative tissue extension after 24 hrs incubation in distilled water for radial tangential tissues sections of transverses slices from the lower, middle and upper stem positions.*

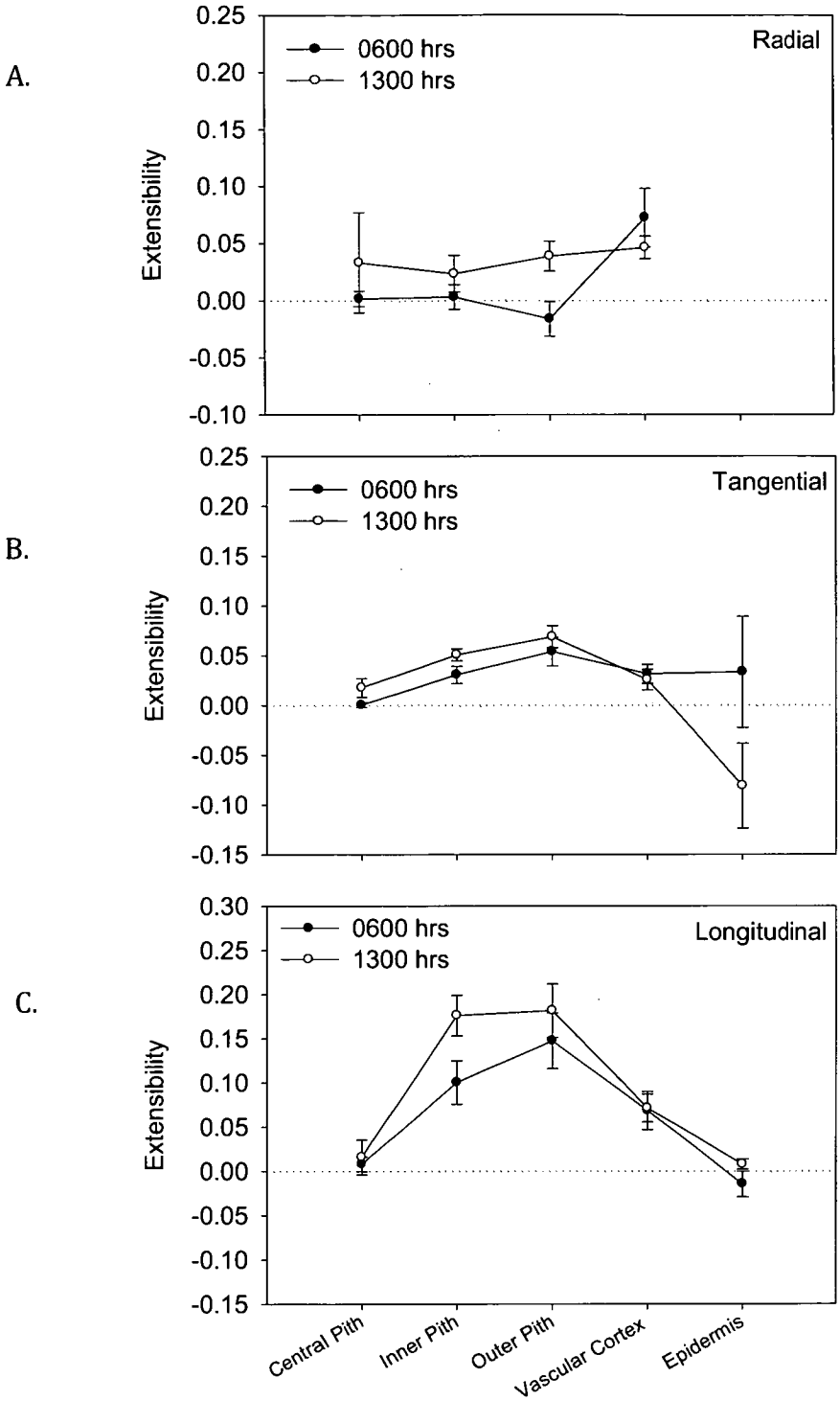


Figure 6. ***(A)*** *Radial,* ***(B)*** *tangential and* ***(C)*** *longitudinal tissue extension from stems sampled at 0600 hrs and 1300 hrs. Data points are the mean relative extension (mean ± SEM) after 24 hrs incubation in distilled water for radial tangential tissues sections of transverses slices pooled across the lower, middle and upper stem positions.*

The time of day at which sampling occurred had no influence on radial extension ($P = 0.48$) nor was there an interaction ($P = 0.092$) between this time of day and tissue type when data for the middle, upper middle and upper stem was pooled (Figure 6A). The different tissues across the stem influenced radial expansion in a pattern similar to that observed in Figure 5A ($P \leq 0.01$), with extensibility being greatest in the vascular / cortex region.

When the tissue of the central pith was placed under radial tensile stress using the Instron material testing machine, tensile strength ($P \leq 0.001$) and breaking strain ($P \leq 0.001$) were significantly different between the three stem positions (Figure 7). Central pith tissue of the lower stem exhibited the highest tensile strength, with the middle and upper stem tissues becoming progressively weaker ($P \leq 0.05$). The lower stem also had significantly higher values for breaking strain ($P \leq 0.05$), than the middle and upper central pith tissues, which were similar. Thus pith tissue in the centre of the lower stem was able to withstand higher tensile stress and was more extensible than the pith tissue in the middle and upper stem regions. While both the middle and upper central pith tissues showed similar properties for extensibility, the tissue located in the upper stem was overall the weakest.

There was a significant interaction ($P \leq 0.001$) between stem position ($P \leq 0.001$) and tissue types ($P \leq 0.001$) for tangential tissue expansion (Figure 5B), however the pattern was different to that of radial extension. In the lower stem, tangential tissue extension was greatest in the central pith tissue and then declined across the outer pith and vascular cortex, with the epidermis showing no extensibility. In the middle and upper stems, tissue extension in the central pith was limited, but then increased from the inner pith to peak in the outer pith tissues before declining slightly in the vascular / cortex region. While the mean tangential expansion of the epidermis appeared higher in the middle stem, this difference was not significant. Thus the epidermal tissue showed little capacity for circumferential expansion in response to hydration.

Tissue expansion in the tangential axis measured in the diurnal trial (Figure 6B) was influenced by type ($P \leq 0.01$), with the pattern here mirroring that previously observed (Figure 5B). Tangential extensibility increased from the central pith to a

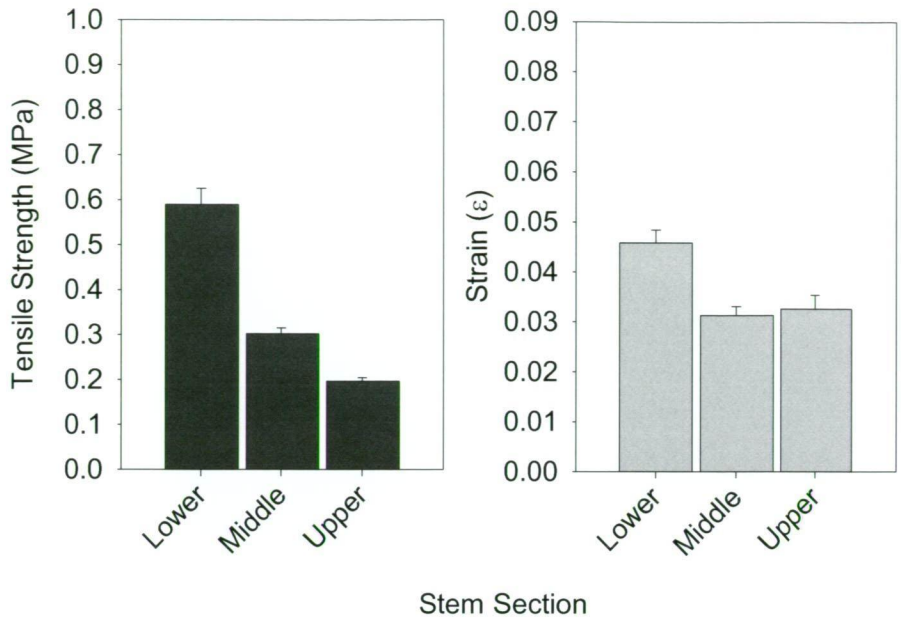


Figure 7. *The tensile strength and strain of the central pith tissues in the lower, middle and upper stem regions.*

peak in the outer pith tissue before dropping slightly in the vascular cortex. The time of day again had no influence on tangential extensibility ($P = 0.357$), except for an interaction with the epidermal tissue ($P \leq 0.05$), indicating some capacity for the epidermis to expand at 0600 hrs, and significant contraction when incubated at 1300 hrs.

Expansion of the lower positioned central pith tissue in the longitudinal dimension produced the greatest relative expansion of all tissues (Figure 5C) and there was a significant interaction ($P \leq 0.001$) between stem position ($P = 0.162$) and tissue type ($P \leq 0.001$). The extensibility observed in the lower stem central pith tissue then declined across the pith tissues being lowest in the epidermis. The pattern of tissue extension in the middle and upper stem positions was similar to that of the tangential axis, being negligible in the central pith tissues and then increasing across the inner pith tissue to a peak in the outer pith. Tissue extensibility then declined in the vascular / cortex tissues, however the decrease was significantly

less in the middle stem position ($P \leq 0.05$). There was only minimal longitudinal extension of the epidermis, with some samples exhibiting contraction.

Both the time of day ($P \leq 0.05$) and tissue type ($P \leq 0.001$) influenced longitudinal extensibility (Figure 6C) however there was no significant interaction ($P = 0.402$). The capacity for the tissues to expand longitudinally was greater at 1300 hrs in the inner and outer pith tissues than at 0600 hrs.

FLUX IN STEM EXPANSION

The rate of increase in the diameter of the upper sections was different between stems (Figure 8) and clear instances of diurnal variation (daytime shrinkage, night time expansion) could be seen on the 11th and 13th June, both days with high vapour pressure deficits. Diurnal variation was greatest in the stem with the highest rate of increase (Stem 3). The upper middle of Stem 1 showed both intraday and diurnal variation (data not shown), but no net increase in diameter (net flux = -0.044 mm). The amplitude of the flux in this section was greater than that of the upper stem on the day samples were collected for the tissue extensibility tests (Figure 9).

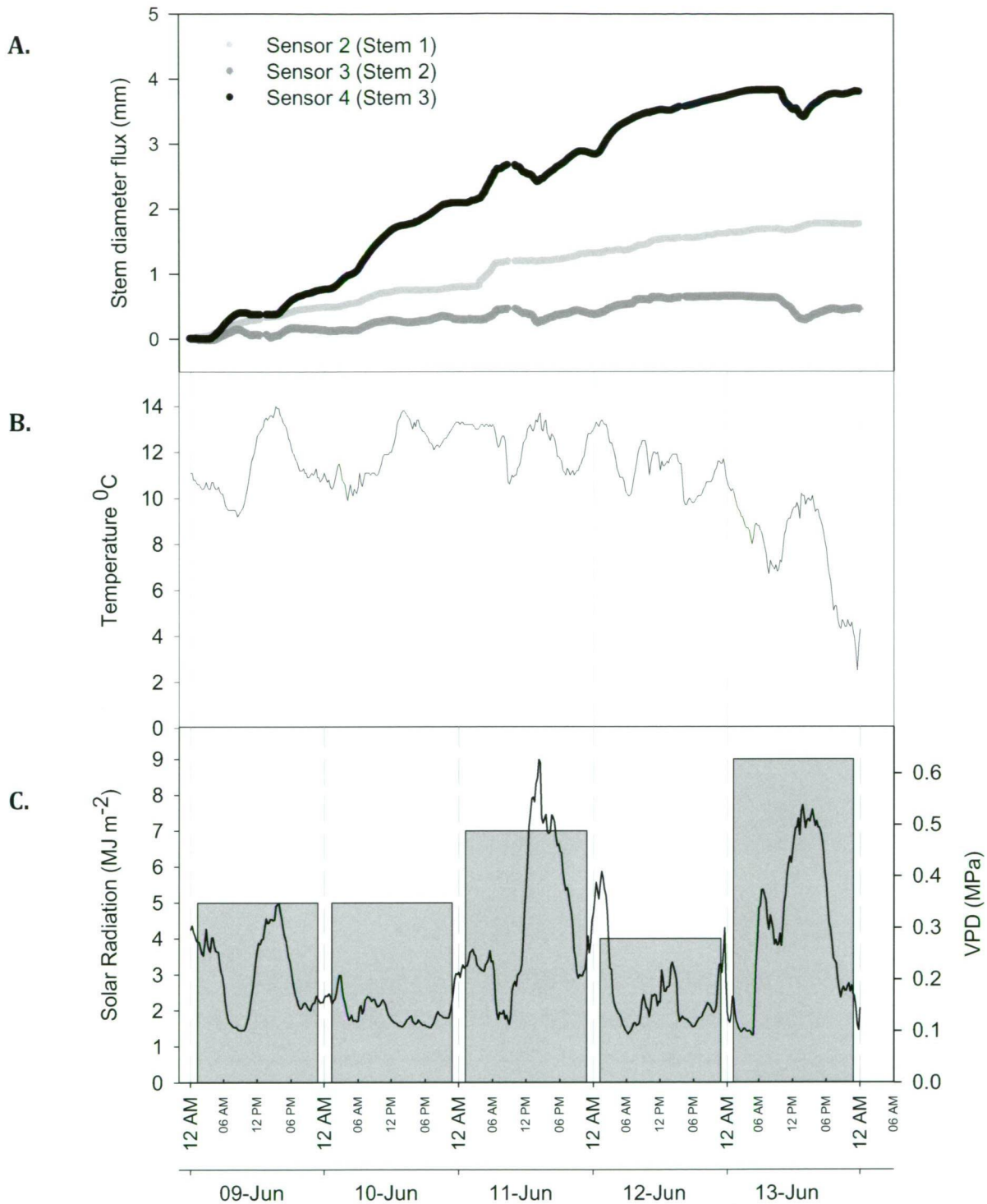


Figure 8. (A) *Flux in stem diameter monitored by sensors placed in the upper stems of three broccoli plants over 5 days.* ***(B)*** *Air temperature and* ***(C)*** *vapour pressure deficit (line) and solar radiation (bars) at Forthside Vegetable Research and Demonstration farm 23 km from the trial site.*

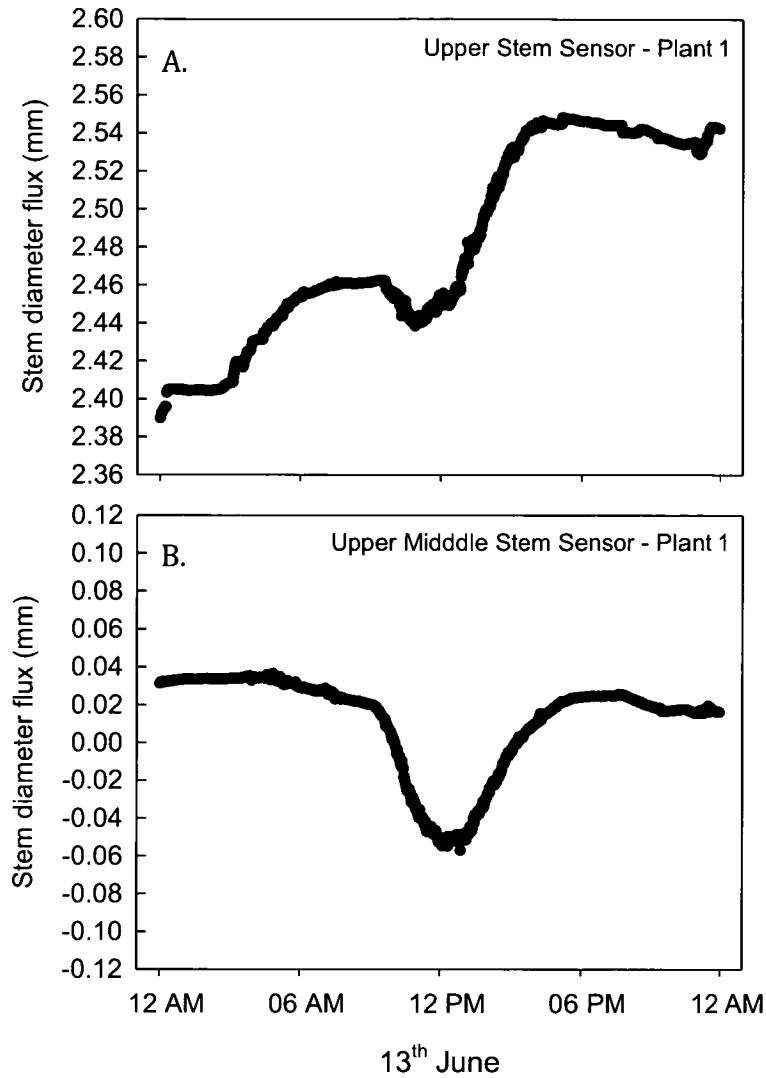


Figure 9. *Diurnal fluctuation in stem diameter on the 13th June at two points on the stem of Plant 1.* ***(A)*** *Sensor 1 located in the upper stem and* ***(B)*** *Sensor 2 located in the upper middle stem.*

DISCUSSION

The de-novo fractures observed during this investigation were similar in appearance to that observed during the early development of hollow stem (Chapter 7). The development of these fractures during hydration provides sound evidence that stresses generated by differential growth across the anisotropic stem tissues is a key mechanism in the generation of hollow stem in broccoli.

For the initiation of the radial longitudinal fracture in the transverse slices of stem to occur, radial strain must have developed within the central pith during hydration of the stem tissues. While the outer pith and vascular cortex tissues of the upper and middle stems showed a significant capacity for radial elastic expansion, this capacity was virtually nonexistent in central and inner pith tissues, and in some samples contraction was observed. It is likely that radial and tangential stress, demonstrated by the extensibility of the inner pith and vascular / cortex tissues, resulted in the circumferential tension stored in the outer ring of tissue comprised of the interfasicular vascular cambium, cortex and epidermal tissues. The capacity for elastic extension of these outer tissues during hydration of the excised tissue ring is also indicative of their capacity to cope with strain generated in this region. The same forces that led to circumferential tension in the outer tissues, may also be responsible for generating radial tensile strain on the less extensible central and inner pith tissues.

The limited extensibility and contraction in the central pith suggests that this tissue had a restricted capacity to expand in response to the strain placed upon it by the annular radial growth occurring in adjacent tissues. Thus stresses created by the annular growth may have generated sufficient radial strain to exceed the strength of the central pith tissue. Once a fracture has been initiated in parenchyma tissue, there is little structural resistance to its propagation (McGarry, 1995), hence they are likely to continue radially and longitudinally throughout the stem until the applied stress is relieved or resisted by other layers of tissue. From this it can be concluded that stem cavities in broccoli can be initiated when radial strain developed by differential growth of the pith tissues exceeds the strength of the central pith tissues, and that this cavity continues to propagate through the stem until the applied stress is resisted or relieved.

The genesis of stem fractures in the top of the stem at inflorescence initiation was a function of the mechanical properties of the central pith tissue in addition to the allometry of growth throughout the stem. During vegetative growth, broccoli stems were tapered at both the base and top of the stem, with the largest increase in diameter occurring in the middle. Once inflorescence development was underway, the upper section of the stem began to undergo a comparatively rapid

expansion in diameter to accommodate the increasing size of the inflorescence. The greater extensibility generated in the outer stem tissues was in effect driving this radial growth. The location in which this annular growth occurred is most likely related to cell division by the vascular cambium. New cells generated here would have the greatest potential to produce the increase in cell volume required to support the radial and tangential tissue expansion necessary for an increase in stem diameter. While this rapid increase in diameter suggests a need for greater extensibility across all tissues, in comparison to the outer stem tissues and the central pith of the lower stem, radial and tangential extensibility was lower in the central pith of the middle and upper section of stem. This, and the lower tensile strength and breaking strain of the central pith, would have made the tissues in this section of the stem more susceptible to failure when subject to radial tensile stress. The growth stresses created by the rapid growth of the upper stem during inflorescence development and the comparative weakness of the central pith tissues in this region may explain why hollow stem begins at the top of the stem in the central pith tissue at this particular growth stage.

The orientation and shape of the elliptical secondary cavities observed to accompany hollow stem (Chapter 7) is suggestive of tissue failure in response to excessive longitudinal strain in the primary cavity walls. If this type of strain does occur it is contradictory to that observed in the hypocotyls of other species such as sunflower, and the hollow stemmed *Reynoutria japonica* Houtt. in which the inner stem tissues are under longitudinal and transverse radial compression (Hejnowicz, 1997). The compression of the inner stem tissues, and equally, tension in the outer stem tissue is a key concept to the much discussed 'epidermal growth control concept' used to explain the upward growth of plants (Kutschera, 2008). As such, strain in the inner parenchyma tissue is contradictory to this theory. The broccoli stem is however comparatively much larger than the 'stems' used in many studies, and the pith tissue much more extensive. In this case it is still possible that sufficient hydrostatic pressure to balance the tensile stress in the epidermal wall was generated by the vascular / cortex tissues and the pith immediately adjacent to the vascular cambium, while allowing strain to develop in the parenchyma tissue of cavities walls.

If strain in the parenchyma tissue of the inner wall of the primary cavity is responsible for secondary elliptical cavities, it may have been generated by upward growth of the plant during inflorescence development (Chapter 7). Enlargement of the inter-nodes could create axial strain in the walls of the primary pith cavity, leading to transverse fractures. For this to occur, the tissue would probably have to exhibit less extensibility than that observed for the inner and outer pith tissues. While these are extensible prior to cavity development, once the initial radial longitudinal fracture has occurred, the mechanical properties of its inner wall tissue may change, possibly making it less extensible. If this is so, the pith tissues forming the inner wall of the cavity may in a sense form an inner skin of less extensible tissue placed under strain via the same mechanism that generates tension in the epidermal wall under the 'epidermal growth control' theory. Additionally, while the outer epidermal tissues have thickened cell walls to accommodate tensile stress (Kutschera, 2008) the thin walled parenchyma do not, making them comparatively more prone to breakage. This scenario does not contradict the basic tenets of the 'epidermal growth control' concept and allows for the development of cavities due to tensile stress in the walls of the primary cavity. The development of longitudinal fractures between these secondary cavities is also evidence of the existence of tangential strain in the wall of the primary cavity (Chapter 7).

The dissimilar types of failure observed in the primary and secondary cavities may be related to the orientation of the cell wall microfibrils in parenchymous tissue, which are typically perpendicular to the axis of elongation (Schopfer, 2006) or helical in nature (Romberger et al., 1993). This orientation of the microfibrils in parenchyma allows cells to elongate while providing sufficient strength necessary to resist the greater transverse stress placed on the cell wall (Schopfer, 2006, Romberger et al., 1993). Cell separation was previously observed in radial longitudinal fractures of young fissures (Chapter 7) and was possibly brought about as radial stress exceeded the strength of the middle lamella. In contrast, the development of the elliptical secondary cavities appears associated with fracture of the cells walls, possibly in response to axial tensile stress (Chapter 7). As the parenchyma cells are reinforced against radial strain, cells in the central pith tissue

may well be more predisposed to separate at the middle lamella than fracture when this type of strain is developed during the genesis of the stem cavity. Conversely, when placed under axial strain during inter-nodal elongation, the longitudinal stress developed within the cell wall in the direction with less microfibrilar resistance, might cause the walls to fracture. Thus the typical orientation of microfibrils within parenchyma tissue might explain the separation of cells under radial strain and fracture under axial strain.

The physical trauma associated with this mechanical damage may in turn initiate a wound response resulting in the creation of necrotic tissue that is observed to accompany hollow stem (Chapter 7, Shattuck et al., 1986).

The different rates of growth and the diurnal variation observed in the upper stems of the plants monitored using the LVDT could possibly explain the plant to plant variability in susceptibility to hollow stem. Different rates of expansion were clearly evident, and under greater rates of radial stem growth, the rate at which strain develops is also likely to increase as the differential between the less extensible central pith tissues and extensible outer pith tissues becomes more pronounced. As the structural characteristics of parenchyma is to a large extent determined by turgor (Romberger et al., 1993) these stresses may also fluctuate in a transient manner due to diurnal variation in transpiration. The stem with the highest growth rate had the greatest diurnal variation and it may be that under these conditions, relatively rapid changes in strain occur. These changes may induce peak loads on the tissues that increase the probability tissue failure, in much the same way as that hypothesised for carrots (Gracie and Brown, 2004). Stems with greater rates of stem expansion and undergoing diurnal variation may therefore be more susceptible to the development of hollow stem.

The development of stem cavities in response to radial expansion of the upper stem can explain the general association between growth rate, plant size and hollow stem observed in other studies (Griffith and Carling, 1991, Zink and Akana, 1951). In studies where growth rate has been increased by lower planting densities or high nitrogen rates, the final width of the stem and most likely the rate of upper stem expansion during inflorescence development would also have

increased. While increased growth rate at low densities is one possible explanation for the increase in hollow stem in broccoli, the increased susceptibility of low density plantings to mechanical perturbation by wind may also play a role in increasing the final stem width (Takano et al., 1995). Broccoli cultivars also vary in their susceptibility to hollow stem (Shattuck et al., 1986) and in this case, small variations in the anatomical structure and arrangement of the different tissue layers may influence the development of stress fractures in the central pith tissue. The mechanical strain hypothesis can accommodate the observed influence of growth rate, plant size and cultivar on the development of hollow stem.

The action of paclobutrazol in reducing the severity of cavity formation (Chapter 8) can be explained by its impact on tissue extensibility. As this compound acts by reducing cell elongation and division it is likely that its application would have reduced the extensibility of the outer stem tissues, lessening both radial and axial strain and thereby limiting the consequent development of severe stem cavities (Rademacher, 2000, Davis and Curry, 1991). While there was no evidence of reduction in the widest stem width measured at the middle of the stem, the increase in stem diameter in the upper stem region was not recorded. However it is in this region that a decrease in the rate of widening of the stem would have been observed. This leaves open the possibility that the action of paclobutrazol in reducing tissue extensibility was responsible for the observed reduction in hollow stem.

Based on these findings and that of previous studies, there are a number of practical approaches that might be taken to reduce the incidence of hollow stem in broccoli. Increasing planting density is an effective tool however there are limitations to this, as the cost of the extra transplants must be balanced against yield, and head size is also decreased at higher population levels (Chung, 1982). Careful nutritional management, particularly with respect to nitrogen application just prior to, or during inflorescence development could potentially be used to tailor stem expansion to levels that minimise the risk of cavity development. Yet it seems likely, that unless tissue structure is being compromised by a nutritional deficiency, fertilizer management that maximises net yield will only at best ameliorate stem cavity development. The use of a plant growth regulator such as

paclobutrazol, uniconazole or others is another option, and could be used independently of growth rate and nitrogen management. The results of the previous study (Chapter 8) also foreshadow considerable efficacy with regard to this use. This avenue is however limited as the application of these compounds in food crops is not an approved use in Australia, and the prospect of this expensive process being undertaken for a minor crop is limited.

While stem mechanics is in all probability a fundamental mechanism, it does not exclude a role for boron deficiency, nor pith autolysis in the development of stem cavities. A sub clinical boron deficiency may for instance predispose the cells to failure as this element is involved in the both cell wall synthesis and structure (Tanaka and Fujiwara, 2008). Pith autolysis as part of a carbohydrate reallocation strategy might also weaken the pith tissue, predisposing it to fracture.

This study has shown that the generation and morphology of stem cavities can be generally explained by differential growth across the stem tissues that exerts radial and longitudinal strain on less extensible pith tissues. The expansion of the upper stem to accommodate an inflorescence of increasing size predisposes this region to cavity formation at this stage of development.

CHAPTER 10

GENERAL DISCUSSION AND FUTURE RESEARCH

The objective of the studies in this thesis was to investigate three areas in which there are biological limitations to improving the net yield and processing efficiency of broccoli. The areas explored included the influence of head shape on processing efficiency and net yield; the pattern of variation in harvest maturity during crop development; and the origin of the quality defect, hollow stem. In each of these areas, new information has been derived that furthers our current understanding of these issues, and has identified opportunities that can be applied or further explored to improve the net yield and processing efficiency of broccoli. In short, these studies have shown that head shape does influence processing efficiency and net yield, and that considerable advantages are endowed by compact inflorescence architecture. A significant proportion of the variation in harvest maturity was found to be introduced during early crop establishment, with the variability during the period of floral initiation being linked to variation in harvest maturity. These studies have also shown that hollow stem, a disorder of which the genesis has remained a mystery since early last century, finds its origin in anisotropic tissue stress generated by differential growth of the tissues in the upper stem. The significance of the discoveries with respect to each of these limitations, and possible areas of future research are explored below.

AN IMPROVED UNDERSTANDING OF HEAD SHAPE

Head shape was found to influence both the net yield and processing efficiency of broccoli. While this outcome is somewhat intuitive, there are no published studies that directly address this issue, with most knowledge of head shape attending to variety selection or the morphological and anatomical development of the

primordial inflorescence. In this investigation, the compact shape of the variety 'Shamrock' was found to produce a higher total floret yield (the most valuable component of the head), and a superior core (stem) to floret (floral branches) ratio when compared to the taller form of 'Marathon'. 'Shamrock' was also found to have a shorter core length when trimmed to the lowest inflorescence branch. As such, heads with compact shapes provide an opportunity to manipulate the amount of the less valuable stem material taken at harvest to suit the seasonal requirements. The rate of floret accumulation with a corresponding increase in head size was also influenced by head shape, being higher in 'Shamrock' than 'Marathon'. Thus delaying harvest in this variety, will result in greater net yields while in 'Marathon', the yield penalty associated with core length will increase at a greater rate. In contrast, 'Marathon', when trimmed to the lowest inflorescence branch, was taller with a more open branch structure. This head form was associated with improved processing efficiency, producing a greater proportion of segments within factory specifications and a smaller proportion that required re-dicing to meet these. These relationships between head shape and processing outcomes in broccoli have not previously been described, and illustrate the important influence head shape can have on net yield and processing efficiency.

In a number of instances, the advantages conferred by shape were postulated to be linked to branch angle. A further exploration of this attribute in future research is warranted as the relationship between branch angle and inter-nodal distance is likely to be an important factor in processing efficiency. An acute angle may be associated with an increased inter-nodal length of the II order or higher branches, as greater elongation would be required to lift the florets to the surface of taller heads. The increase in length of these branches may subsequently contribute to a comparatively open branch structure that facilitates a higher degree of segmentation, as observed for 'Marathon'. If inter-nodal distance is related to branch angle, then the factors driving this will also be of interest. Primordial development of the branches ceases once the higher order meristems assume floral identity, and changes to the inter-nodal distances may be effected by the relative rate of primordia production or the iteration interval (Kieffer et al., 1998, Chapter 3). Equally, elongation of the internodes could be altered by increased cell

elongation in longer internodes during curd development after primordial development has ceased. Branch angle is also likely to determine core length, as heads of similar diameter but with a more acute angle will necessarily have longer cores when cut to the lowest inflorescence branch.

While the radial orientation of the primordial inflorescence branches in a spiral phyllotaxis is determined by genetics (Hardwick, 1984), other architectural attributes such as branch angle may be influenced by environmental factors. This is true for other plant organs, where the quantity and quality of light can influence both leaf angle, inter-nodal distances and the timing of floral initiation (Smith, 1997, Whitelam and Johnson, 1982). The lack of variability across sites observed for branch angle within the variety 'Marathon' does however suggest that this aspect of head shape is tightly controlled by plant genetics. As such further work is required to elucidate the influence of both genetic and environmental factors on the architecture of the developing inflorescence, particularly with respect to branch angle. An increased understanding of these relationships will allow for improved cultivar selection, and if influenced by the external environment, direct changes in agronomic processes.

Establishing a relationship between the branch order and position at which a segment is cut and its dimensions would also be of benefit. For instance, if it was determined that segmentation through the III order branches produced segments of more appropriate size, the blade trajectory could be altered to meet this requirement, or alternatively, attempts could be made to alter the inter-nodal distances of the higher order branches. This relationship between branch order and segment size is likely to be complicated by the reduced size of the metamers in the higher positions of the main axis.

While environment appears to have some influence, the high within site variation observed in the descriptive attributes of 'Marathon' suggests that expression of the floral genes could vary within a variety. It also raises the possibility that some varieties may be more stable in the expression of head shape than others. By utilising the head shape parameters used in this study and the relationships established between heads shape and processing outcomes, selection of future

varieties for processing could also be enhanced. The data from this study suggests that selection should favour moderately compact head shapes with a short core length and relatively low branching angles, and an eccentricity matching that of the processing machinery (e = 0.75). As the architectural attributes of 'Shamrock' conferred poorer processing efficiencies, it maybe that the ideal head shape is less compact than this variety. As both diameter and height of the inflorescence increase together, to maximise the segmentation and yield of compact heads, plants with a compact inflorescence should be grown out to the greatest diameter possible. This will maximise the net floret yield and will additionally improve segmentation through the increased elongation of both the core and II+ order branches.

This study has contributed new insights and highlights the considerable impact that head shape can have on the net yield and processing efficiency of broccoli. It has also provided a foundation from which further research into plant varieties and agronomic practices can be used to manage the core:floret ratio and improve processing efficiency and net yield by manipulating the size distribution of the floret segments produced.

AN IMPROVED UNDERSTANDING OF HARVEST MATURITY

The results of this investigation point to the timing of floral initiation as an important influence on variation in harvest maturity, and that floral evocation is occurring around the time of transplanting. As a consequence of this, the transplanting process and conditions during establishment are likely protagonists in the variability observed in the progress towards flowering of broccoli. In this study meristem diameter was used as a measure of a plants progress towards floral initiation, and as a measure of development during curd growth. Variation in broccoli's progress in inflorescence development was found to increase from 28 DAP, and continued to increase until the late stages of curd development. However, harvest maturity, when measured as head size, was linked to the variability in meristem diameter during the early period of increase at 28 DAP. As most plants were transitioning at this stage it was concluded that evocation had occurred prior

to this time. A logistic regression between plant size and the transition stage also revealed that 50% of the plants in the study had reached transition between 14- 28 DAP. Meristem diameter also increased rapidly from transplanting onwards, this again indicating that evocation had occurred at or near the time of transplanting (Wurr et al., 1995). Collectively, this provides plausible evidence that a considerable proportion of the variability in harvest maturity is introduced at the time of floral initiation. The variability in floral initiation is in turn likely to be influenced by factors that modify the competency individual plants to respond to evocation stimuli, such as the conditions experienced during transplant and establishment, when evocation is occurring.

These conclusions are however restricted to some extent by the existing deficiencies in the definition of harvest maturity. Head size is currently the most reliable descriptor, as others such as maximum bead size, evenness of bud size or colour (Chung, 1982, Chowings, 1974), are either heavily influenced by other variables such as variety, or in the case of bead compactness, hard to quantify with precision. Additionally, maturity in the context of current descriptions, is often described in terms of upper limits, for example a maximum bead size (Chung, 1982), while under maturity can often be hard to define. While head size is probably the most suitable gross measure of maturity, this too is influenced by factors such as physiological development. As the heads approach physiological maturity, the rate of increase in head size is likely to decrease, and probably cease altogether once elongation of the II order branches begins just prior to the opening of the floral beads. Thus, although not observed in this study, within a population head size may become increasingly skewed as physiological maturity approaches. The development of a scale to rate the physiological progress of flowering past the stages currently described (Tan et al., 1998) would allow for the precise stage of maturity to be identified in future studies. This type of scale has been developed for *Arabidopsis thaliana* L. Heynh (Smyth et al., 1990) and could be adapted with relative ease to describe floret development in broccoli, as these plants are of the same botanical family. The development of this scale would provide a basis for the precise and repeatable measure of broccoli maturity, allowing for a better comparison of treatments both within and between studies.

This study has however provided sufficient evidence to warrant the consideration of future research focussing on transplanting practices and the environment during establishment, and specifically, the influence of these factors on the timing of floral initiation. There are a least two major variables that could conceivably alter the plants flowering response during this period, seedling damage, and adverse soil conditions that disrupt homeostasis of the plants water potential. Damage to the seedlings during transplanting could possibly delay or halt the response to vernalisation as has been observed for cotyledon damage in other broad leaf species (Hanley and Fegan, 2007). Similarly, soil moisture prior to, and after transplanting may also influence seedling uniformity, as would moisture of the root plug itself (Wien, 1997). These factors are influenced by watering of both the seedling plugs and field prior to transplanting, and by irrigation of the field after transplanting. Identification of the influence of moisture content in each of these situations on floral initiation would allow for specific recommendations on irrigation practices that maximise the uniformity of floral initiation and harvest maturity.

The total variation in harvest maturity is comprised of both variation in environmental factors and the underlying variation introduced by plant genetics. This genetic component may be viewed as more significant, as the uniform phenological expression of the genes associated with flowering is foundational to uniform harvest maturity. This is because the variability contributed by the genetic component in a sense provides 'background' variation that is then compounded by environmental factors. Thus while efforts can be made to improve on agronomic practices that influence floral initiation, the role of plant breeding in this process is also of importance. Broccoli cultivars have been shown to vary in this respect and previous studies have shown that when modelling the response to thermal time, the cultivar 'Marathon' is innately variable in the timing of floral initiation when compared to other cultivars (Tan et al., 2000a). Given this, varietal studies examining the variability in floral initiation between cultivars under uniform environmental conditions could identify those with less variability and greater stability in the expression of floral phenology.

While the data from this study indicates that floral evocation is occurring close to the time of transplanting, to maximise uniformity in floral initiation, a more precise estimate of the transition between the juvenile and vegetative phases is necessary. A more accurate estimation of this phase change would allow for a better understanding of the impact agronomic practices may be having on the variability encountered in floral initiation. Molecular biology techniques are rapidly unravelling the genetic control of flowering in a number of species, including broccoli, and it is likely that these techniques can be used to precisely identify the timing of juvenile and vegetative phases. Once this is understood, the precise timing of floral initiation in relation to transplanting and establishment can be determined and the influence of agronomic practices could then be tailored to minimise their influence on this variable.

This study has increased the current understanding of the variability in harvest maturity by elucidating the pattern in which variability in the broccoli's progress towards flowering is introduced, and linking the variability in meristem diameter at transition to variability in harvest maturity when measured as head size. The evidence provided can be used to infer that future research to reduce the number of picking events required to fully harvest a crop, should focus on varietal selection and the agronomic practices employed during transplanting and establishment.

AN IMPROVED UNDERSTANDING OF HOLLOW STEM

The occurrence of stem cavities in broccoli has been sporadically investigated since early last century and its cause has been variously attributed to either boron deficiency or growth rate related factors. While previous descriptions of the disorder have been largely based on boron deficiency studies, this dissertation has provided a detailed description of hollow stem in field grown plants under adequate boron supply. While stem cavity formation was a common symptom between this study and previous boron deficiency studies, many of the symptoms previously described for boron deficiency were not observed. While stem cavity formation undoubtedly occurs in response to boron deficiency (Shelp et al., 1992, Petracek and Sams, 1987), the lack of other associated symptoms suggested the

possibility that stem cavities may also occur in response to other separate mechanisms. One such alternative mechanism is mechanical tissue strain. The investigations in this study have also provided strong evidence that the development of stem cavities is driven by mechanical strain generated across the stem tissues. While the role of radial strain within the stem as a protagonist has previously been postulated (Griffith and Carling, 1991) this is the first study to provide evidence that this is indeed a key mechanism.

In the hydration studies used here, tissue in the outer pith and the vascular / cortex region were found to be more extensible than the central pith tissues in the middle and upper stem sections of broccoli. Significant circumferential strain was also stored in the vascular / cortex tissues indicating the extensibility of the outer tissues is generating both radial and tangential growth stresses within the stem. It is hypothesised that these stresses are generating radial strain on the less extensible pith tissues at the centre of the stem, exceeding its mechanical limits. The subsequent failure of these tissues results in the initiation of a radial longitudinal fracture. This fracture is then propagated longitudinally and radially throughout the parenchyma tissue, possibly via cell wall separation through the middle lamella, until resisted by different tissue layers or the strain is released. The patterning of tissue extensibility in the lower stem is opposite to that of the middle and upper stem, and the breaking strength and strain of the central pith tissue is also greatest here, making it less susceptible to fracture. Accordingly, the pattern of tissue extensibility in the upper stem in conjunction with the necessary radial expansion required to accommodate the increasing size of the inflorescence, provides a sound explanation for the occurrence of stem cavities in the upper stem at this stage of growth. The data generated in this study and the accompanying stem mechanics hypothesis has improved on the current understanding of the causal mechanism of hollow stem, in addition to providing a focus for future research efforts.

The stem mechanics hypothesis can explain the association of hollow stem with factors such as lower planting densities and high nitrogen applications in addition to other treatments that promote growth rate. These factors would be likely to increase the rate of growth and / or the final size of the upper stem, thus

increasing the chances that radial strain will exceed the strength of the tissues. However as the expansion or final size of the upper stem is not commonly measured, it is difficult to show this conclusively in retrospect. While providing an adequate explanation for stem fracture in broccoli, this hypothesis does not rule out the possibility that a sub clinical boron deficiency may still play a role in the development of stem cavities. Boron is involved in cell wall synthesis and structure (Tanaka and Fujiwara, 2008) and therefore it is still possible that a deficiency in this element could predispose the central pith tissues to failure. There is however little evidence to support this viewpoint. Akin to this, it can also be postulated that programmed cell death as part of carbohydrate redistribution (Carr and Jaffe, 1995) during inflorescence development may also make the central pith tissue more susceptible to failure under radial strain, particularly if this involves autolysis of the tissues via programmed cell death. This hypothesis could be explored in future research through ultrastructural and immunohistochemical studies of the central pith tissues just prior to the development of stem cavities.

In this study the extension of the various tissues is interpreted as potential for growth, with different magnitudes of extensibility representing the differential capacity of each tissue section to undergo growth. However, the measurement of the expansion of these tissues is only representative of elastic expansion, and not plastic growth, as the reversibility of this response was not measured (Hejnowicz, 1997). Yet the inability of the central pith tissues to expand elastically in a hypotonic solution does suggest that the cells comprising this tissue are inherently different in their capacity for irreversible cell expansion.

The explanation for the differential pattern in extensibility of the various stem tissues is likely to be identified using histological studies. In the absence of detailed studies on the cellular composition of the various tissues found throughout broccoli stems, tissues in this study were only classified at a gross level. However, even within the pith which is typically comprised of thin walled parenchyma cells, there are likely to be differences in cell types in this region of the broccoli stem. For example, at a macroscopic level the white inner pith tissue is distinct in colour from the green tissue of the inner and outer pith, suggesting that these outer tissues are possibly chlorenchyma. A histological study detailing the different type

of cells and their associated mechanical attributes (i.e. the location of collenchyma) could further contribute to explaining the differences in extensibility and mechanical properties observed across these tissues, particularly if this is combined with ultrastructural studies of the cell walls. Future studies should also consider ontogeny, as the characteristics of these tissues might also change with plant age. The position of these tissues within the stem should also be considered as this study has indicated differences in extensibility according to the longitudinal position within the stem.

During the handling of the specimens in this work the central pith tissues appeared more brittle than those of the outer stem, suggesting that this tissue has a lower breaking strain than the outer tissues. Therefore a more strict classification and excision of the different tissue types would also provide an opportunity to further explore the mechanical properties of each layer based on cell type, providing further insight into the interaction between growth induced stress and the inherent mechanical properties of the tissues themselves.

The orientation of the major transverse axis of the main cavity also appeared to be perpendicular to the major axis of the upper stem, which is typically elliptical rather than circular in cross section. Elliptical growth requires greater radial expansion of the tissues on the stems major transverse radial axis, and less on the minor axis. This imbalance in growth rate may in turn determine the orientation of the initial fracture, with its major axis being perpendicular to that of the stems. Confirmation of this arrangement would provide further circumstantial evidence of the role of stem tissue stress in the development of stem cavities.

The identification of mechanical stress as a key mechanism in the development of stem cavities also provides new opportunities to explore the management of this disorder in a commercial context. The application of paclobutrazol was observed to significantly decrease the incidence of hollow stem, probably because of it role in reducing cell expansion (Rademacher, 2000). Thus plant growth regulators (PGR) applied at inflorescence initiation might be used to reduce the incidence of this disorder. There are some challenges to this approach for while PGR's are registered for use in crops, there are currently no registrations for this use in

vegetables. For this to occur, the compounds must go through the expensive process of proving them safe for use in this situation. The expense associated with this would likely be prohibitive for a minor crop such as broccoli. Using these compounds to manipulate stem growth rates should however prove a useful tool to further our understanding of stem cavity development.

Careful management of crop growth rate may be another avenue through which the levels of hollow stem can be minimised. The use of higher planting densities and the judicious use of fertiliser could conceivably be used to grow crops at a rate that does not lead to the development of severe stem cavities while minimising any associated reduction in yield. The pursuit of this opportunity would however require further research to determine firstly if this is possible, and secondly establish the appropriate planting density and pattern of fertiliser application. Alternatively, crop growth might be manipulated by growing in locations or seasons in which ambient temperatures might slow growth of the upper stem during inflorescence development.

Of more immediate relevance is the opportunity to review the current application of boron trace elements which are regularly applied to broccoli crops in Tasmania, on the understanding that this is likely to mitigate the development of hollow stem. Given that the weight of literature suggests the hollow stem development in field grown broccoli is not related to a boron deficiency, and the strong evidence suggesting a central role played by stem mechanics in the development of hollow stem, it is likely that many of these applications are unnecessary. As such it is recommended that boron trace elements need only be applied in cases where soil boron levels are below the critical nutrient range applicable to broccoli. The application of this advice will reduce the extra costs associated with the application of this trace element.

This dissertation has provided a new hypothesis supported by convincing evidence that stress generated across the anisotropic stem tissues results in radial strain on the less extensible and weaker central pith tissues, leading its failure and the initiation of an initial radial longitudinal fracture that later enlarges to form a stem cavity. The advancement of this hypothesis has opened up new opportunities to

further explore the intricacies of this disorders development in addition to providing opportunities to research alternative agronomic management tools.

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